

186 Human Secreted Proteins

This application claims benefit under 35 U.S.C. 119(e) from U.S. Provisional Patent Application Serial No. 60/190,068; and this application is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of copending United States patent application Serial No. 09/149,476, filed September 8, 1998, which is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of copending International Application No. PCT/US98/04493, filed March 6, 1998, which is hereby incorporated herein by reference, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Applications:

	Filing Date:	Application
1.	07-Mar-1997	60/040,162
2.	07-Mar-1997	60/040,333
3.	07-Mar-1997	60/038,621
4.	07-Mar-1997	60/040,626
5.	07-Mar-1997	60/040,334
6.	07-Mar-1997	60/040,336
7.	07-Mar-1997	60/040,163
8.	23-May-1997	60/047,600
9.	23-May-1997	60/047,615
10.	23-May-1997	60/047,597
11.	23-May-1997	60/047,502
12.	23-May-1997	60/047,633
13.	23-May-1997	60/047,583
14.	23-May-1997	60/047,617
15.	23-May-1997	60/047,618
16.	23-May-1997	60/047,503
17.	23-May-1997	60/047,592
18.	23-May-1997	60/047,581
19.	23-May-1997	60/047,584
20.	23-May-1997	60/047,500
21.	23-May-1997	60/047,587
22.	23-May-1997	60/047,492
23.	23-May-1997	60/047,598
24.	23-May-1997	60/047,613
25.	23-May-1997	60/047,582
26.	23-May-1997	60/047,596
27.	23-May-1997	60/047,612
28.	23-May-1997	60/047,632
29.	23-May-1997	60/047,601
30.	11-Apr-1997	60/043,580
31.	11-Apr-1997	60/043,568
32.	11-Apr-1997	60/043,314
33.	11-Apr-1997	60/043,569
34.	11-Apr-1997	60/043,311
35.	11-Apr-1997	60/043,671

"03T90" T/28860

36.	11-Apr-1997	60/043,674
37.	11-Apr-1997	60/043,669
38.	11-Apr-1997	60/043,312
39.	11-Apr-1997	60/043,313
40.	11-Apr-1997	60/043,672
41.	11-Apr-1997	60/043,315
42.	06-Jun-1997	60/048,974
43.	22-Aug-1997	60/056,886
44.	22-Aug-1997	60/056,877
45.	22-Aug-1997	60/056,889
46.	22-Aug-1997	60/056,893
47.	22-Aug-1997	60/056,630
48.	22-Aug-1997	60/056,878
49.	22-Aug-1997	60/056,662
50.	22-Aug-1997	60/056,872
51.	22-Aug-1997	60/056,882
52.	22-Aug-1997	60/056,637
53.	22-Aug-1997	60/056,903
54.	22-Aug-1997	60/056,888
55.	22-Aug-1997	60/056,879
56.	22-Aug-1997	60/056,880
57.	22-Aug-1997	60/056,894
58.	22-Aug-1997	60/056,911
59.	22-Aug-1997	60/056,636
60.	22-Aug-1997	60/056,874
61.	22-Aug-1997	60/056,910
62.	22-Aug-1997	60/056,864
63.	22-Aug-1997	60/056,631
64.	22-Aug-1997	60/056,845
65.	22-Aug-1997	60/056,892
66.	22-Aug-1997	60/057,761
67.	23-May-1997	60/047,595
68.	23-May-1997	60/047,599
69.	23-May-1997	60/047,588
70.	23-May-1997	60/047,585
71.	23-May-1997	60/047,586
72.	23-May-1997	60/047,590
73.	23-May-1997	60/047,594
74.	23-May-1997	60/047,589
75.	23-May-1997	60/047,593
76.	23-May-1997	60/047,614
77.	11-Apr-1997	60/043,578
78.	11-Apr-1997	60/043,576
79.	23-May-1997	60/047,501
80.	11-Apr-1997	60/043,670
81.	22-Aug-1997	60/056,632
82.	22-Aug-1997	60/056,664
83.	22-Aug-1997	60/056,876
84.	22-Aug-1997	60/056,881
85.	22-Aug-1997	60/056,909
86.	22-Aug-1997	60/056,875

09082171 "051201"

87.	22-Aug-1997	60/056,862
88.	22-Aug-1997	60/056,887
89.	22-Aug-1997	60/056,908
90.	06-Jun-1997	60/048,964
91.	05-Sep-1997	60/057,650
92.	22-Aug-1997	60/056,884
93.	05-Sep-1997	60/057,669
94.	13-Jun-1997	60/049,610
95.	02-Oct-1997	60/061,660
96.	08-Jul-1997	60/051,926
97.	16-Jul-1997	60/052,874
98.	12-Sep-1997	60/058,785
99.	18-Aug-1997	60/055,724

09882171 051304
"000000" 000000

Field of the Invention

This invention relates to newly identified polynucleotides, polypeptides encoded by these polynucleotides, antibodies that bind these polypeptides, uses of such polynucleotides, polypeptides, and antibodies, and their production.

5

Background of the Invention

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

10

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

15

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

20

25

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of

30

FOIA b 7 - DATED 06-13-01 BY 60321 JCF

the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the

ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

5 A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC
10 (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions.

15 Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl;
20 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

25 Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking
30 reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA⁺ sequences (such as any 3' terminal polyA⁺ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of

ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

This gene is expressed primarily in testes tumor and to a lesser extent in fetal brain.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer particularly of the testes, and defects of the central nervous system such as seizure and neurodegenerative disorders. Similarly, polypeptides and
10 antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly cancer of the testes and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. testes and other reproductive
15 tissue, brain and other tissue of the nervous system, and blood cells, and spleen, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not
20 having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment/diagnosis of testicular cancer and the treatment of central nervous system disorders since this gene is primarily expressed in the testes tumor and developing brain. Alternatively, expression within
25 fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or
30 tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1005 of SEQ ID NO:11, b is an integer of 15 to 1019, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 2

This gene is expressed primarily in cancer tissues, such as breast cancer, Wilm's tumor, and to a lesser extent in fetal tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tumors, particularly, those found in the breast, or developmental abnormalities or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the glandular tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. mammary tissue, fetal tissue, developmental tissue, and cancerous and wounded tissues) or bodily fluids (e.g. breast milk, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in breast cancer cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment/diagnosis of

cancers and/or tumors, particularly, those found in the breast since expression is mainly in cancer/tumor tissues. Similarly, expression within embryonic tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 321 as residues: Pro-11 to Thr-18, Leu-43 to Pro-50, Gly-64 to Leu-72, Leu-81 to Lys-86.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 451 of SEQ ID NO:12, b is an integer of 15 to 465, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 3

The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed primarily in CD34 depleted buffy coat, and to a lesser extent in spleen, chronic lymphocytic leukemia.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: blood disorders or leukemias, diseases of the immune or hematopoietic system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, and spleen, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Expression of this gene product in spleen and leukemia cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood

lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 460 of SEQ ID NO:13, b is an integer of 15 to 474, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

This gene is expressed primarily in CD34 depleted buffy coat.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or hematopoietic disorders, particularly lymphocytic diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, and cancerous and wounded tissues) or bodily fluids (e.g.lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 depleted buffy coat suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic

09882171.061801

related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 300 of SEQ ID NO:14, b is an integer of 15 to 314, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

This gene is expressed primarily in CD34 depleted buffy coat.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at

significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 depleted buffy coat suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 324 as residues: Pro-13 to Lys-21.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 599 of SEQ ID NO:15, b is an integer of 15 to 613, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

This gene is expressed primarily in CD34 depleted buffy coat.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 318 as residues: Lys-31 to Lys-39.

The tissue distribution in CD34 depleted buffy coat suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 325 as residues: Lys-31 to Lys-39.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 342 of SEQ ID NO:16, b is an integer of 15 to 356, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

This gene is expressed primarily in CD34 depleted buffy coat, and to a lesser extent in the pineal gland.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune, hematopoietic, neural, or endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, blood cells, endocrine, neural, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 depleted buffy coat suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic

lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, expression within the pineal gland suggests that the protein product of this clone would be useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism), hypothalamus, and testes. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 400 of SEQ ID NO:17, b is an integer of 15 to 414, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

The translation product of this gene shares sequence homology with an organic cation transporter which is thought to be important in organic cation uptake in the kidney and liver. (See Accession No. 2343059.) Preferred polypeptide fragments comprise the amino acid sequence

ITIAIQMICLVNXELYPTFVRNXGVMVCSSLCDIGGIITPFIVFRL
 REVWQALPLILFAVLGLLAAGVTLLLPETKGVALPETMKDAENLGRKAKPKE
 NTIYLKVQTSEPSGT (SEQ ID NO: 629) or TMKDAENLGRKAKPKENT (SEQ
 ID NO: 630) as well as N-terminal and C-terminal deletions of these fragments. Also
 5 preferred are polynucleotide fragments encoding these polypeptide fragments.

This gene is expressed primarily in liver.

Therefore, polynucleotides and polypeptides of the invention are useful as
 reagents for differential identification of the tissue(s) or cell type(s) present in a
 biological sample and for diagnosis of diseases and conditions: hepatic and renal
 10 diseases where drug elimination/cation exchange (organic cation uptake) in the liver
 and kidney are problematic. Similarly, polypeptides and antibodies directed to these
 polypeptides are useful in providing immunological probes for differential
 identification of the tissue(s) or cell type(s). For a number of disorders of the above
 tissues or cells, particularly of the hepatic or renal system, expression of this gene at
 15 significantly higher or lower levels may be routinely detected in certain tissues and
 cell types (e.g., kidney and liver, and cancerous and wounded tissues) or bodily fluids
 (e.g. bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell
 sample taken from an individual having such a disorder, relative to the standard gene
 expression level, i.e., the expression level in healthy tissue or bodily fluid from an
 20 individual not having the disorder.

The tissue distribution and homology to organic cation transporter indicate
 that polynucleotides and polypeptides corresponding to this gene are useful as a
 polyspecific transporter that is important for drug elimination in the liver (and
 possibly kidney) since expression is found in the liver. Similarly, the tissue
 25 distribution in liver suggests that the protein product of this clone would be useful for
 the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma,
 jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the
 differentiation of hepatocyte progenitor cells). In addition the expression in fetus
 would suggest a useful role for the protein product in developmental abnormalities,
 30 fetal deficiencies, pre-natal disorders and various would-healing models and/or tissue
 trauma. Moreover, the homology to the organic ion transporter may suggest that this
 gene or gene product could be used in the treatment and/or detection of kidney

diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 327 as residues: Asn-64 to Asn-74, Gln-81 to Gly-87.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 455 of SEQ ID NO:18, b is an integer of 15 to 469, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

This gene is expressed primarily in eosinophils induced with IL-5, and to a lesser extent in fetal liver and spleen. The gene encoding the disclosed cDNA is believed to reside on chromosome 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: diseases of the immune system, particularly allergies or asthma, in addition to hepatic, or developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential

identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, liver, and spleen, and cancerous and wounded tissues) or
 5 bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides
 10 corresponding to this gene are useful for treating/diagnosis of diseases involving eosinophil reactions since expression seems to be concentrated in eosinophils and other tissues involved in immunity such as the liver and spleen. Similarly, expression within embryonic tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and
 15 may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility
 20 as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
 25 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 536 of SEQ ID NO:19, b is an integer of 15 to 550, where both a and b correspond to the positions of
 30 nucleotide residues shown in SEQ ID NO:19, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

This gene is expressed primarily in tissues of hematopoietic lineage and to a lesser extent in Hodgkins lymphoma. Any frame shifts in this sequence can easily be clarified using known molecular biology techniques.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for the diagnosis of diseases and conditions which include, but are not limited to, immune deficiencies or dysfunctions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., hematopoietic cells, lymphoid and reticuloendothelial tissues, and cancerous tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment/ diagnosis for lymphomas or immune dysfunction or as a therapeutic protein useful in immune modulation based on expression in anergic T-cells and lymphomas. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 18 as residues: Gly-6 to Asp-7, Pro-20 to Ser-21, Ser-23 to Cys-24, Arg-26 to Arg-26.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 759 of SEQ ID NO:20, b is an integer of 15 to 773, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 11

This gene is expressed primarily in neutrophils and to a lesser extent in activated lymphoid cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the cell type present in a biological sample and for diagnosis of diseases and conditions: immune or hematopoietic disorders, particularly inflammatory conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells and lymphoid tissue, immune, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia,

pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed

progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 330 as residues: Glu-40 to Lys-46.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 977 of SEQ ID NO:21, b
15 is an integer of 15 to 991, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

20 This gene is expressed primarily in brain and to a lesser extent in activated T-cells. It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. Preferred polypeptide fragments comprise the amino acid sequence PRVRNSPEDLGLSLTGDCKL (SEQ ID NO:631).

Therefore, polynucleotides and polypeptides of the invention are useful as
25 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or neurodegenerative disorders including ischemic shock, alzheimers and cognitive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the
30 tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g.,

blood cells, brain, other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system.

Alternatively, the tissue distribution in activated T-cells suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Expression of this gene product in tonsils suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as

autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 331 as residues: Ser-5 to Glu-14, Ile-21 to Pro-35, Ser-65 to Asp-81, Cys-89 to Val-96, Lys-136 to Ser-145, Ile-152 to Met-169, Arg-189 to Lys-196.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 639 of SEQ ID NO:22, b is an integer of 15 to 653, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

This gene was also recently cloned by other groups, naming this calcium-activated potassium channel gene, hKCa4. (See Accession No. AF033021, see also, Accession No. 2584866.) This gene is mapped to human chromosome 19q13.2. A second signal sequence likely exists upstream from the predicted signal sequence as described in Table 1. Preferred polypeptide fragments comprise:

QADDLQATVAALCVLRGGGPWAGSWLSPKTPGAM

GGDLVLGLGALRRRKRL (SEQ NO: 632); or

EQEKSLAGWALVLAXXGIGLMVLH

AEMLWFGGCSAVNATGHLSDTLWLPITFLTIGYGDVVPGTMWVGKIVCLCTG
VMG

VCCTALLVAVVARKLEFNKAEKHVHNFMMDIQYTKEMKESAARVLQEAW
MFYKHTRRKESHAARXHQRXLLAAINAFRQVRLKHRKLREQVNSMVDISKM

5 HMILYDLQQNLSSSHRALEKQIDTLAGKLDALTELLSTALGPRQLPEPSQQSK
(SEQ ID NO: 633), as well as N-terminal and C-terminal deletions. Also preferred are
polynucleotide fragments encoding these polypeptide fragments.

This gene is expressed primarily in breast lymph node and T-cells, and to a
lesser extent in placenta.

10 Therefore, polynucleotides and polypeptides of the invention are useful as
reagents for differential identification of the tissue(s) or cell type(s) present in a
biological sample and for diagnosis of diseases and conditions: hematologic, immune,
reproductive, or developmental disorders. Similarly, polypeptides and antibodies
directed to these polypeptides are useful in providing immunological probes for
15 differential identification of the tissue(s) or cell type(s). For a number of disorders of
the above tissues or cells, particularly of the immune or reproductive system,
expression of this gene at significantly higher or lower levels may be routinely
detected in certain tissues and cell types (e.g., lymphoid tissue, blood cells and
placenta, neural, and cancerous and wounded tissues) or bodily fluids (e.g. lymph,
20 amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue
or cell sample taken from an individual having such a disorder, relative to the
standard gene expression level, i.e., the expression level in healthy tissue or bodily
fluid from an individual not having the disorder. Preferred epitopes include those
comprising a sequence shown in SEQ ID NO. 325 as residues: Arg-13 to Lys-23.

25 The tissue distribution suggests that the protein product of this clone would be
useful for the diagnosis and treatment of a variety of immune system disorders.
Expression of this gene product in tonsils suggests a role in the regulation of the
proliferation; survival; differentiation; and/or activation of potentially all
hematopoietic cell lineages, including blood stem cells. This gene product may be
30 involved in the regulation of cytokine production, antigen presentation, or other
processes that may also suggest a usefulness in the treatment of cancer (e.g. by
boosting immune responses). Since the gene is expressed in cells of lymphoid origin,

the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, expression within placental tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 332 as residues: Arg-13 to Lys-23.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1472 of SEQ ID NO:23, b is an integer of 15 to 1486, where both a and b correspond to the positions of

09882171-061803
FOI b7D b7C b7E b7F

nucleotide residues shown in SEQ ID NO:23, and where the b is greater than or equal to a + 14.

09882171-061801
"08F90" FZF28860

FEATURES OF PROTEIN ENCODED BY GENE NO: 14

The translation product of this gene was found to have homology with the human PAPS synthase, which is believed to be involved in modification of L-selectin ligands (See Accession No. e1204135.) Preferred polypeptide fragments

5 comprise the amino acid sequence

YQAAHVSRNKRQVVGTRGGFRGCTVWLTGLSGAGK (SEQ ID NO: 634),
 SPFLLPGEVPASRGGSGPSPFSFSLRIDVLPPPPPSRVLRSLLPGPSAQP
 ASMSGIKKQKTENQQKSTNVVYQAAHVSRNKRQVVGTRGGFRGCTVWLT
 G

10 LSGAGKNNDKFCPGGVLVSHAIPVNSWMGTMSVMALTESPRWLHGPQSME
 GPDRLQLQVPAEELSLWSRVSF (SEQ ID NO: 635), VLPWRSTCLPCHPC (SEQ
 ID NO: 636), or FLDGDNVRHGLNRIPQMASWPPKHGRS (SEQ ID NO: 637).
 Also preferred are the polynucleotide fragments encoding this polypeptide fragment.

It has been discovered that this gene is expressed primarily in benign prostate
 15 hyperplasia, human umbilical vein endothelial cells and to a lesser extent in smooth
 muscle and human endometrial stromal cells-treated with estradiol.

Therefore, polynucleotides and polypeptides of the invention are useful as
 reagents for differential identification of the tissue(s) or cell type(s) present in a
 biological sample and for diagnosis of diseases and conditions: inflammation, ischemia,
 20 and restenosis, based on endothelial cell and smooth muscle cell expression, and
 prostate diseases such as benign prostate hyperplasia or prostate cancer. Similarly,
 polypeptides and antibodies directed to these polypeptides are useful in providing
 immunological probes for differential identification of the tissue(s) or cell type(s). For
 a number of disorders of the above tissues or cells, particularly of the prostate or
 25 vessels of the circulatory system, expression of this gene at significantly higher or
 lower levels may be routinely detected in certain tissues and cell types (e.g., prostate,
 endothelial cells, smooth muscle, and endometrium, and cancerous and wounded
 tissues) or bodily fluids (e.g. seminal fluid, serum, plasma, urine, synovial fluid or
 spinal fluid) or another tissue or cell sample taken from an individual having such a
 30 disorder, relative to the standard gene expression level, i.e., the expression level in
 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating/diagnosing diseases or conditions where the endothelial cell lining of the veins and arteries of underlying smooth muscle are involved. Alternatively, expression within prostate tissue suggests that the protein product of this clone may show utility in the study, detection, treatment, or prevention of a variety of reproductive disorders, particularly prostate cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 333 as residues: Arg-21 to Asp-26, Lys-35 to Lys-44, Glu-49 to Asn-58.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2309 of SEQ ID NO:24, b is an integer of 15 to 2323, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 15

This gene is expressed primarily in human 6 week embryo, and to a lesser extent in placenta.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: reproductive disorders, particularly developmental anomalies or fetal deficiencies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly reproductive or

09882171.061801

developmental in nature, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., embryonic tissue, developing, differentiating, placenta, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution within human embryonic, placental, and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 334 as residues: Lys-50 to Glu-57.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 669 of SEQ ID NO:25, b is an integer of 15 to 683, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

This gene is expressed primarily in kidney, amygdala, and to a lesser extent in fetal tissues. The gene encoding the disclosed cDNA is believed to reside on chromosome 14. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 14.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) present in a biological sample and for diagnosis of diseases and conditions: kidney diseases, neurological disorders and developmental abnormalities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s). For a number of disorders of the above tissues, particularly of the renal system or developing fetal tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., kidney, amygdala, and fetal tissues, and cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in kidney suggests that this gene or gene product could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Similarly, expression within neural tissue suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In

addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2022 of SEQ ID NO:26, b is an integer of 15 to 2036, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 17

This gene is expressed primarily in ovarian cancer.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: reproductive disorders, particularly solid tumors similar to ovarian cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system. expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., ovarian and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment of solid tumors of the reproductive system such as ovarian cancer. Similarly, expression within cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 336 as residues: Ser-51 to Val-56.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 703 of SEQ ID NO:27, b is an integer of 15 to 717, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 18

This gene is expressed primarily in brain medulloblastoma. Preferred polypeptide fragments comprise the amino acid sequence:

IRHEQHPNFSLEMHSKGSSLLLFLPQL ILILPVCAHLHEELNC (SEQ ID NO: 638) and SFFISEEKGHLLLQAERHPWVAGA

LVGVSGGLTLTTCSGPTEKPATKNYFLKRLQEMHIRAN (SEQ ID NO: 639), as well as N-terminal and C-terminal deletions. Also preferred are polynucleotide fragments encoding these polypeptide fragments.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural disorders, particularly tumors of the CNS. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the Central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Similarly, expression within cellular sources marked by

0992171.051801
T08T90.T28860

proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 481 of SEQ ID NO:28, b is an integer of 15 to 495, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

This gene is expressed primarily in adipocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: obesity. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the adipose tissues expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., adipocytes and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative

to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating metabolic disorders, particularly obesity by regulating the function and number of adipocytes. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 542 of SEQ ID NO:29, b is an integer of 15 to 556, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where the b is greater than or equal to a + 14.

20 **FEATURES OF PROTEIN ENCODED BY GENE NO: 20**

This gene is expressed primarily in B cell lymphoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, the immune system with an emphasis on B cell lymphoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the tumors of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, and lymphoid tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken

from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders, particularly for diagnosis and treatment of B cell derived tumors. Expression of this gene product in B-cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 420 of SEQ ID NO:30, b is an integer of 15 to 434, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where the b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 21

It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. Therefore, in specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

10 TRPISQLRHYCEPYTTWCQE

TYSQTKPK (SEQ ID NO: 640). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in immune cells, and to a lesser extent in fetal tissues

15

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or reproductive disorders, particularly inflammatory or developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., cells of the immune system, and fetal tissues, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25

The tissue distribution suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Expression of this gene product in immune cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all

30

09882171 051801

hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 340 as residues: Asp-10 to Pro-19, Ser-74 to Tyr-79, Glu-95 to Lys-110.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 701 of SEQ ID NO:31, b is an integer of 15 to 715, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 22

It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. Therefore, in specific embodiments, polypeptides of the invention comprise the following amino acid sequence: SQCRRKGTFLYFLPQTLS PHTSCPCSAGRPLPPPVL DSTPSSPSN (SEQ ID NO: 641). Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been discovered that this gene is expressed primarily in ovarian tumor.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: reproductive or endocrine disorders, particularly tumors of the ovary. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of tumors of the reproductive organs. expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., ovarian and other reproductive tissue and cancerous and wounded tissues) or bodily fluids (e.g.lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, treatment, and/or prevention of a variety of reproductive or endocrine disorders, particularly ovarian. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 341 as residues: Leu-22 to Gln-27.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 472 of SEQ ID NO:32, b is an integer of 15 to 486, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where the b is greater than or equal to a + 14.

15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 23**

The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

It has been discovered that this gene is expressed primarily in fetal tissues, and to a lesser extent in osteoclastoma cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: osteoporosis or arthritis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., bone cells, fetal tissue, developmental, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative

to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment of conditions of a variety of skeletal disorders, particularly abnormal bone remodeling due to enhanced activity of osteoclasts. This may be useful as a specific marker for malignancies derived from osteoclasts or their precursors. Alternatively, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 711 of SEQ ID NO:33, b is an integer of 15 to 725, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 24

The translation product of this gene shares sequence homology with a periplasmic ribonuclease which is thought to be important in degrading extracellular polynucleotides. In specific embodiments, polypeptides of the invention comprise the

following amino acid sequence:

HSMLPSDVSYLYHMKTLTLLQLQDTERLKHLEMFPEHCTMPPAFIGSCR
NQIGRSSVPAAPNVEKYRSIPKEPTPMTWTQESYNLRGLFPSVHCRAHATHH
L

- 5 ACPDPRXATPCDNSR (SEQ ID NO: 642). Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been discovered that this gene is expressed primarily in serum treated smooth muscle cells

- Therefore, polynucleotides and polypeptides of the invention are useful as
10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: vascular disease such as restenosis, atherosclerosis, stroke, or aneurysm. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of
15 disorders of the above tissues or cells, particularly of the vasculature expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., smooth muscle, vascular, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a
20 disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- The tissue distribution in smooth muscle and homology to ribonucleases indicate that polynucleotides and polypeptides corresponding to this gene are useful for treatment of pathological conditions of smooth muscle associated with bacterial or
25 viral infiltration. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 343 as residues: Gln-30 to Lys-36, Pro-41 to Arg-48.

- 30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
 5 general formula of a-b, where a is any integer between 1 to 423 of SEQ ID NO:34, b is an integer of 15 to 437, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where the b is greater than or equal to a + 14.

10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 25**

This gene is expressed primarily in early stage human brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural or
 15 developmental disorders, particularly neurodegenerative or behavioral disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the human brain development and related diseases, expression of this gene at significantly
 20 higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, developmental, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the
 25 expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease,
 30 Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms,

hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or

5 detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Alternatively, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative

10 disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 344 as residues: Pro-20 to Glu-25.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of

20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 929 of SEQ ID NO:35, b

25 is an integer of 15 to 943, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 26

30 It has been discovered that this gene is expressed primarily in human brain tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural disorders, particularly brain diseases, such as neurodegenerative disorders which may be caused by brain diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the human brain diseases, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 345 as residues: Glu-31 to Leu-36.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 590 of SEQ ID NO:36, b is an integer of 15 to 604, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 27

It has been discovered that this gene is expressed primarily in anergic T-cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune diseases, particularly inflammatory diseases and diseases related to T lymph cells, such as immunodeficiencies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune diseases, inflammatory diseases and diseases related to T lymph cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders.

Expression of this gene product in T-cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be

involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 346 as residues: Gly-26 to Ser-35.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 335 of SEQ ID NO:37, b is an integer of 15 to 349, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 28

The translation product of this gene shares sequence homology with *Shigella flexneri* positive transcriptional regulator CriR (criR) gene which part of a two-component regulatory pathway and is thought to be important in regulation of gene expression. Since there are multiple examples of prokaryotic two-component

5 regulatory pathways that have significant parallels to signal transduction pathways in eukaryotic tissues, it is anticipated that this gene would have utility for treating human disorders. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: LLHELVQAH

YPGDVVFTTAASDMETVSEAVRCGVFDYLIKPIAYXXLGQTLTRFRQRKHML

10 ESID

SASQKQIDEMFNAYARGEPKDELPTGIEPLTLNAVRKLFXPGVQHTAXTVX
QALNHQPHHCQALS (SEQ ID NO: 643), or

TISRTTARRYLEYCASRHLIAEIVHGKVGR PQRIYHSG (SEQ ID NO: 644).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in human synovial sarcoma and normal human brain tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: human brain diseases

20 particularly sarcomas of the synovium. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the human brain and synovium and other related human brain diseases, expression of this gene at significantly higher or lower
25 levels may be routinely detected in certain tissues and cell types (e.g., synovial tissue, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or
30 bodily fluid from an individual not having the disorder.

The tissue distribution in brain, combined with the homology to a two-component regulatory protein suggests that the protein product of this clone would be

useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 658 of SEQ ID NO:38, b is an integer of 15 to 672, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 29

The gene encoding the disclosed cDNA is believed to reside on the X chromosome. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for the X chromosome.

This gene is expressed in bone marrow, infant brain, fetal liver and spleen, prostate and to a lesser extent in pineal gland, adipose tissue, kidney, adrenal gland, umbilical vein endothelial cells, and T cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: diseases related to bone marrow or hematoplastic tissues, prostate, kidney, neural, adrenal gland, and cardiovascular tissue or organs. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the diseases related to hematoplastic tissues, immune system, prostate, kidney, adrenal gland, and cardiovascular tissue or organs, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., bone marrow, hematopoietic cells, pineal gland, adipose tissue, neural, developing, kidney, adrenal gland, endothelial cells, and blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to the gene indicate that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of diseases related to hematoplastic tissues, immune system, prostate, kidney, adrenal gland, and cardiovascular tissue or organs. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1894 of SEQ ID NO:39, b is an integer of 15 to 1908, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:39, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 30

5 In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

MKISFVDLPKSIVVTRPSQNLSSFTVQPCVLGPQPNNSIVPHF

TFHGQLPSLLDIKPHILNXVEI (SEQ ID NO: 645). Polynucleotides encoding these polypeptides are also encompassed by the invention.

10 This gene is expressed primarily in meningeal and to a lesser extent in breast and adult brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural disorders, particularly diseases of the meningea and related brain diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the meningea and related brain diseases, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., miningeal, mammary tissue, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25 The tissue distribution in meningeal tissue suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive

0988271.051801

disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo,

5 sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 349 as residues: Ser-19 to Glu-26.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 444 of SEQ ID NO:40, b is an integer of 15 to 458, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where the b is greater than or equal

20 to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 31

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in

25 linkage analysis for chromosome 12.

This gene is expressed in meningea, fetal spleen, osteoblast and to a lesser extent in activated T-cells, endometrial stromal cells, fetal lung, HL-60, thymus, testis and endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as

30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: meningeal disease, osteoporosis, immune diseases, and hematoplastic diseases. Similarly, polypeptides

and antibodies directed to these polypeptides are useful in providing immunological probes for identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the meningeal diseases, osteoporosis, immune diseases, and hematoplastic diseases, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, endometrium, lung, thymus, testis, and endothelial cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of meningeal, osteoporosis, immune diseases, hematoplastic diseases, reproductive or pulmonary disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 350 as residues: Gln-7 to Arg-12, Pro-69 to Glu-76, Leu-119 to Trp-125.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1139 of SEQ ID NO:41, b is an integer of 15 to 1153, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 32

The translation product of this gene was shown to have homology to the bovine leucine aminopeptidase (LAP), which is thought to be involved in the intracellular degradation and turn-over of cellular proteins (See Genbank Accession No. bbs|137417). In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: PQFTSAGENF (SEQ ID NO: 646),

5 LKAGKTRTFYGL (SEQ ID NO: 647),

AGIDEQENWHEGKENIRAAVAAGCRQIQDLE (SEQ ID NO: 648),

SVEVDPCGDAQ (SEQ ID NO: 649), EPPLVFVGKGITFDSGGISIK (SEQ ID NO: 650), ANMDLMRAD

10 MGGAATICSIVSAAKL (SEQ ID NO: 651),

GLAPLCENMPSPGKANKPGDVVRA (SEQ ID NO: 652),

NGKTIQVDNTDAEGRLLADALCYAHTFNPK (SEQ ID NO: 653),

ALGSGATGVFTNSSWLWNKLFEASITGDRVWRMPLFEHYTRQV (SEQ ID NO: 654),

15 VNNIGKYRSAGACTAAAFLEFVTHPKWAHLDIAGVMTNKDEVPLYLRKGM

AAAEGLVGLYEYDDLKQK (SEQ ID NO: 655), or DMTKGLVLGIYSKEKE (SEQ ID NO: 656). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human thymus and to a much lesser extent

20 in infant brain, T-cells, smooth muscle, endothelial cells, bone marrow, human ovarian tumor and keratinocytes testes, osteoclastoma, breast, and tonsils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: Diseases involving the

25 thymus, particularly thymic cancer and diseases involving T-cell maturation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the thymus, expression of this gene at significantly higher or lower levels may be

30 routinely detected in certain tissues and cell types (e.g., thymus, brain, and other tissue of the nervous system, blood cells, bone marrow, ovaries, and testes, and other reproductive tissue, mammary tissue, tonsils, melanocytes and cancerous and

wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 The tissue distribution in thymus and immune tissues, combined with the homology to the LAP gene indicate that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of diseases of the thymus particularly thymic cancer and diseases involving T-cell maturation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

10 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 351 as residues: Ser-42 to Val-49, Lys-79 to Thr-85, Asp-109 to Asn-120, Asp-163 to Lys-170, Tyr-178 to Trp-186, Pro-206 to Pro-212, His-265 to Glu-274, Met-338 to Gly-347, Asp-361 to Glu-366.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1969 of SEQ ID NO:42, b is an integer of 15 to 1983, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 33

This gene is expressed primarily in human tonsils, and placenta, and to a lesser extent in adipocytes, melanocyte, and infant brain.

30 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: inflammatory

diseases, immune diseases, and metabolic disorders, particularly obesity. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the inflammatory diseases, immune diseases, and obesity, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., metabolic tissue, tonsils, placenta, adipocytes, melanocytes, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to this gene indicate that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of diseases such as inflammation, immune diseases, and obesity. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 352 as residues: Ser-26 to Ser-34.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1392 of SEQ ID NO:43, b is an integer of 15 to 1406, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 34

The translation product of this gene was found to have homology to the human

FGF-1 intracellular binding protein which is thought to play a role in the regulation of fibroblast growth factors (See Genbank Accession No. gi|2738520 (AF010187)). In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

MTSELDIFVGNTTLIDEDVYRLWLDGYSVTDVAVALRVRSGILEQTG
ATAAVLQSDTMDHY (SEQ ID NO: 657),

RTFHMLERLLHAPPKLLHQLIFQIPPSR

QALLIERYYAFDEAFVREVLGKKLSKGTKKDL (SEQ ID NO: 658),

DDISTKTGITLK

SCRRQFDNFKRVFKVVEEMRGSLVDNIQQHFLLSDRLARDYAAIVFFA (SEQ
ID NO: 659), NNRFETGKKKLQYLSFGDFAFCAELMIQNWTLG (SEQ ID NO:

660), AAKLTHNKDVRDLFVDLV (SEQ ID NO: 661),

EKFVEPCRS DHWPLSDVRFFLNQ

YSASV (SEQ ID NO: 662), SLDGFRHQA (SEQ ID NO: 663), RPPTLTIKLL (SEQ
ID NO: 664), IMTEVPPN (SEQ ID NO: 665), or

ISIQRLSNPSMASDTRPSGTATWAPS

AAASCACIMTEVPPNVRPR (SEQ ID NO: 666). Polynucleotides encoding these polypeptides are also encompassed by the invention. When tested against Jurkat T-cell cell

lines, supernatants removed from cells containing this gene activated the GAS (gamma activation site) promoter element. Thus, it is likely that this gene activates T-cells through the Jaks-STAT signal transduction pathway. GAS is a promoter element found upstream in many genes which are involved in the Jaks-STAT pathway. The Jaks-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells. The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly,

polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed in activated T cells, and to a lesser extent in pituitary, testis, and breast lymph node.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders, particularly diseases relating to T-cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for
10 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the disorders of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, hematopoietic, pituitary, testes and other reproductive tissue, mammary tissue, and lymphoid tissue, and cancerous
15 and wounded tissues) or bodily fluids (e.g. lymph, seminal fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 The tissue distribution in T-cells and lymph nodes, combined with its homology to a known regulatory protein for FGF-1, and the detected GAS activity in T-cells, strongly suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Specifically, the expression of this gene product in T-cells suggests a role in the regulation of the
25 proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin,
30 the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis,

granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense
 5 tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the
 10 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 353 as residues: Lys-111 to Thr-125, Ser-133 to Lys-142, Asn-182 to Lys-190, Asp-216 to Asp-221, Asp-227 to Thr-233.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
 20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1377 of SEQ ID NO:44, b is an integer of 15 to 1391, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where the b is greater than or equal
 25 to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 35

This gene is expressed primarily in infant brain.

Therefore, polynucleotides and polypeptides of the invention are useful as
 30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural disorders, particularly neurodegenerative or behavioral disorders. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the diseases relating to neurological disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain, and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in infant brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 354 as residues: Ser-67 to Glu-74, Ala-117 to Leu-126, Gln-128 to Arg-137, Lys-158 to Gly-167.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

09882171.051801

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1555 of SEQ ID NO:45, b is an integer of 15 to 1569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 36

This gene is expressed primarily in infant brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the diseases relating to neurological disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or

09882171.061801

detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Alternatively, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:46 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1910 of SEQ ID NO:46, b is an integer of 15 to 1924, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 37

Polynucleotides of the invention do not comprise the nucleic acid sequence shown as Genbank accession no. gb|G22195|G22195, which is hereby incorporated herein by reference. The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in human ovary.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: ovarian cancer.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the ovarian disorders such as those involving germ cells, ovarian follicles, stromal cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., ovary and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, treatment, and/or prevention of a variety of reproductive or endocrine disorders, particularly ovarioopathies, tumors, or dysfunctions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 356 as residues: Met-1 to Gly-17, Glu-49 to Ile-54.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 461 of SEQ ID NO:47, b is an integer of 15 to 475, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 38

This gene is expressed primarily in lymph node breast cancer.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune or reproductive disorders, particularly breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the breast cancer, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., mammary tissue and lymphoid tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for use as a diagnostic marker for breast cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 357 as residues: Leu-16 to Asp-24.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 332 of SEQ ID NO:48, b is an integer of 15 to 346, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 39

The translation product of this gene was shown to have homology to the type III adenylyl cyclase of *Rattus norvegicus* (See Genbank Accession No gi|202715.), which is thought to play an essential role in mediating signal transduction in the sensory neuronal cilia of olfactory neurons. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

ERWQHLADLADFALAMKDTLTNNNQSFN (SEQ ID NO: 667), or

HFLHGPLAQEDKSERERWQHLADLADFALAMKDTLTNN

NQSFNXHC (SEQ ID NO: 668). Polynucleotides encoding these polypeptides

are also encompassed by the invention.

This gene is expressed primarily in brain, and to a lesser extent in other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neuronal disorders such as trauma, brain degeneration, and brain tumor. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms,

hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or

5 detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.

10 358 as residues: Gln-19 to Phe-25.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

15 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1352 of SEQ ID NO:49, b is an integer of 15 to 1366, where both a and b correspond to the positions of

20 nucleotide residues shown in SEQ ID NO:49, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 40

Polynucleotides of the invention do not comprise the nucleic acid sequence

25 shown as Genbank accession no. gb|AB006624|AB006624, which is hereby incorporated herein by reference.

This gene is expressed in early stage human embryo, adrenal gland tumor, and immune tissues such as fetal liver, fetal spleen, T-cell, and myeloid progenitor cell line and to a lesser extent in ovary, colon cancer, and a few other tissues.

30 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: tumorigenesis

including adrenal gland tumor, colon cancer and various other tumors, developmental and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cancer tissues, early stage human tissues, and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, spleen, blood cells, developmental, bone marrow, ovary and other reproductive tissue, and colon, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Alternatively, the tissue distribution in myeloid progenitor cells, in addition to fetal spleen, suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the

protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1391 of SEQ ID NO:50, b is an integer of 15 to 1405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 41

This gene is expressed primarily in fetal lung, endothelial cells, liver, thymus and a few other immune tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders such as immune deficiency and autoimmune diseases, pulmonary diseases, liver diseases, developmental disorders, and tumor metastasis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal lung, liver, endothelial cells, and immune tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., lung, endothelial cells, liver, thymus, and other tissue of the immune system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, pulmonary surfactant, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard

gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis of immune disorders and pulmonary and hepatic diseases. Its promoter may also be used for immune system and lung-specific gene therapies. The expression of this gene in endothelial cells indicates that it may also involve in angiogenesis which therefore may play role in tumor metastasis. Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2619 of SEQ ID NO:51, b is an integer of 15 to 2633, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 42

This gene is expressed primarily in liver, thyroid, parathyroid and to a lesser extent in fetal lung, stomach and early embryos.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: metabolic regulation, obesity, hepatic failure, hepatocellular tumors, endocrine disorders, particularly thyroiditis and thyroid tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive/endocrine system expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, thyroid, parathyroid, lung, stomach, and embryonic tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, bile, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and the extracellular locations indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection and treatment of digestive/endocrine disorders, including metabolic regulation, hepatic failure, malabsorption, gastritis and neoplasms. Similarly, expression within thyroid and parathyroid tissues suggests that the protein product of this clone would be useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism), hypothalamus, and testes. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

09882171.061801

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 763 of SEQ ID NO:52, b is an integer of 15 to 777, where both a and b correspond to the positions of
5 nucleotide residues shown in SEQ ID NO:52, and where the b is greater than or equal to a + 14.

09882171.061801

FEATURES OF PROTEIN ENCODED BY GENE NO: 43

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

5 This gene is expressed primarily in Schizophrenic adult brain, pituitary, front cortex, hypothalamus and to a lesser extent in retina, adipose and stomach cancer and placenta.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: schizophrenia and other neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. neural, retinal tissue, adipose, stomach, and placenta, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful in treatment/detection of disorders in the nerve system, including schizophrenia, neurodegeneration, and neoplasia. Additionally, a secreted protein in brain may serve as an endocrine. Similarly, the secreted protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anaemia or as adjunct to chemotherapy);

stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating haemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behaviour. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 362 as residues: Pro-14 to Ser-23, Ser-57 to Phe-65, Asn-121 to Asn-131.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 588 of SEQ ID NO:53, b is an integer of 15 to 602, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 44

The translation product of this gene shares sequence homology with a human GTP binding protein which are thought to be important in signal transduction and protein transport (See Genbank Accession No. dbj||D84488_1). In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

MGSRDHLFKVLVVGDAAVGKTSLVQDYSQDSFSKHYKSTVGVDFAKVLQ
WSDYEIVRLQ (SEQ ID NO: 669), LWDIAGQERFTSMTRLYYRDASACVIMFD

VTNATTFSNSQRWKQDLDSKLTLPNGEPVPC (SEQ ID NO: 670), LLANKCD
LSPWAVSRDQIDRFSKENGFTGWTETSVKENKNINEAMRVLIEKMMRNSTED
(SEQ ID NO: 671), or IMSLSTQGDYINLQTK (SEQ ID NO: 672). Polynucleotides
encoding these polypeptides are also encompassed by the invention.

5 This gene is expressed primarily in umbilical vein and microvascular
endothelial cells, GM-CSF treated macrophage, anergic T cells, osteoblast, osteoclast,
CD34+ cells and to a lesser extent in gall bladder.

Therefore, polynucleotides and polypeptides of the invention are useful as
reagents for differential identification of the tissue(s) or cell type(s) present in a
10 biological sample and for diagnosis of diseases and conditions: bone formation and
growth, osteonecrosis, osteoporosis, angiogenesis and/or hematopoiesis. Similarly,
polypeptides and antibodies directed to these polypeptides are useful in providing
immunological probes for differential identification of the tissue(s) or cell type(s). For
a number of disorders of the above tissues or cells, particularly of the skeletal and
15 hematopoiesis systems, expression of this gene at significantly higher or lower levels
may be routinely detected in certain tissues and cell types (e.g., endothelial cells,
blood cells, bone, and gall bladder, and cancerous and wounded tissues) or bodily
fluids (e.g. lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid or spinal
fluid) or another tissue or cell sample taken from an individual having such a
20 disorder, relative to the standard gene expression level, i.e., the expression level in
healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in various immune tissues and cell types combined
with its homology to a conserved GTP binding protein indicates that polynucleotides
and polypeptides corresponding to this gene are useful for treatment/detection of bone
25 formation and growth, osteonecrosis, osteoporosis, and/or hematopoiesis because its
involvement in the growth signaling or angiogenesis. Protein, as well as, antibodies
directed against the protein may show utility as a tumor marker and/or
immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
30 363 as residues: Asp-26 to Ser-38, Leu-76 to Ala-81, Ser-97 to Ser-108, Asp-137 to
Phe-148, Thr-154 to Ile-162, Gln-194 to Ser-199.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1735 of SEQ ID NO:54, b is an integer of 15 to 1749, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 45

The translation product of this gene shares sequence homology with a signal sequence receptor gamma subunit which is thought to be important in protein translocation on the endoplasmic reticulum.

This gene is expressed primarily in adrenal gland, salivary gland, prostate, and to a lesser extent in endothelial cells and smooth muscle.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: endocrine or gastrointestinal disorders, particularly disorders in protein secretion. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the secretory organs, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., adrenal gland, salivary gland, prostate, endothelial cells, and smooth muscle, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in various endocrine tissues and homology to SSR gamma subunit indicate that polynucleotides and polypeptides corresponding to this gene are useful for endocrine disorders, prostate cancer, xerostomia or sialorrhea. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 364 as residues: Lys-27 to Ser-32.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1882 of SEQ ID NO:55, b is an integer of 15 to 1896, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where the b is greater than or equal to a + 14.

20 FEATURES OF PROTEIN ENCODED BY GENE NO: 46

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in osteoclastoma cells, and to a lesser extent in melanocyte, amygdala, brain, and stomach.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: skeletal disorders, particularly ossification, osteoporosis, fracture, osteonecrosis, osteosarcoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the

09882171.061801

skeletal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. skeletal, melanocytes, amygdala, brain and other tissue of the nervous system, and stomach, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in osteoclastoma cells suggests that the protein product of this clone may play a role in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis as well as disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (ie. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. indicates that polynucleotides and polypeptides corresponding to this gene are useful in intervention of ossification, osteoporosis, fracture, osteonecrosis and osteosarcoma.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 365 as residues: Gln-19 to Tyr-27, Pro-47 to Glu-59.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:56 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1739 of SEQ ID NO:56, b is an integer of 15 to 1753, where both a and b correspond to the positions of

T 08190 T 228860

nucleotide residues shown in SEQ ID NO:56, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 48

It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. Therefore, in specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

RACRHSIYSAWVTSVNITD

CKPPSISGAAHQGPAPGRMVRLANGEIVQDDDPVRVTTTQPPRGSIPRQSFF

NRGHGAPPGGPGPRQQQAGARLGAAQSPFNDLNRQLVN (SEQ ID NO: 673).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 10.

Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

The translation product of this gene shares sequence homology with proline rich proteins which is thought to be important in protein-protein interactions.

This gene is expressed primarily in brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neurological and psychological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nerve system and endocrine system,

expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to proline-rich proteins indicate that polynucleotides and polypeptides corresponding to this gene are useful in intervention and detection of neurological diseases, including trauma, neoplasia, degenerative or metabolic conditions in the central nerve system. Additionally, the gene product may be a secreted by the brain as an endocrine. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1035 of SEQ ID NO:58, b is an integer of 15 to 1049, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 49

The translation product of this gene shares sequence homology with the AOCB gene from *Aspergillus nidulans* which is important in asexual development. The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

This gene is expressed primarily in infant brain and to a lesser extent in the developing embryo, trachea tumors, B-cell lymphoma and synovial sarcoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neurodegenerative diseases, leukemia and sarcoma's. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential

identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., embryonic tissue, neural, blood cells, trachea, and synovial tissue, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in infant brain and sarcoma's and homology to a gene involved in a key step of eukaryotic development (fungal spore formation) indicates that the protein product of this clone could play a role in neurological diseases such as schizophrenia, particularly in infants. The existence of the gene in a B-cell lymphoma indicates the gene may be used in the treatment and detection of leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 368 as residues: Thr-8 to Glu-13, Thr-89 to Leu-96, Ser-144 to Leu-152, Arg-160 to Asp-166.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1762 of SEQ ID NO:59, b is an integer of 15 to 1776, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 50

This gene is expressed primarily in fetal lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: pulmonary disorders including lung cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the pulmonary system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., lung, developing, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, pulmonary surfactant, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene only in fetal lung indicates that it plays a key role in development of the pulmonary system. This would suggest that misregulation of the expression of this protein product in the adult could lead to lymphoma or sarcoma formation, particularly in the lung. It may also be involved in predisposition to certain pulmonary defects such as pulmonary edema and embolism, bronchitis and cystic fibrosis. Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:60 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 429 of SEQ ID NO:60, b is an integer of 15 to 443, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where the b is greater than or equal to a + 14.

10 FEATURES OF PROTEIN ENCODED BY GENE NO: 51

The gene encoding the disclosed cDNA is believed to reside on chromosome 22. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 22.

It has been discovered that this gene is expressed primarily in hematopoietic cell types and fetal cells and to a lesser extent in all tissue types.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: defects in the immune system and hematopoiesis. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution of this gene predominantly in hematopoietic cells and in the developing embryo suggests that the protein product of this clone would be useful for the detection and treatment of lymphomas and disease states affecting the immune system or hematopoiesis disorders. Expression of this gene product in hematopoietic cells suggests a role in the regulation of the proliferation; survival;

differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:61 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2874 of SEQ ID NO:61, b is an integer of 15 to 2888, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 52

It has been discovered that this gene is expressed primarily in prostate and to a lesser extent in fetal spleen, fetal liver, infant brain and T cell leukemias.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: prostate disorders, prostate cancer, leukemia. Similarly, polypeptides and antibodies directed to those polypeptides are

useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, and/or prostate gland expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution of this gene in prostate suggests that the protein product of this clone would be useful for detection or treatment of prostate disorders or prostate cancer. Its distribution in fetal liver and fetal spleen suggests it may play a role in the immune system. Expression of this gene product in hematopoietic cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 371 as residues: Gln-70 to Asn-80, Leu-129 to Gly-150, Pro-153 to Thr-166, Met-174 to Val-181, Thr-185 to Ser-196, Pro-200 to Glu-216, Phe-225 to Ser-230, Ile-232 to Lys-253, Cys-257 to Pro-271, Leu-293 to Pro-298, Ile-339 to Asn-344.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:62 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1837 of SEQ ID NO:62, b is an integer of 15 to 1851, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 53

The translation product of this gene shares sequence homology with dynein.

It has been discovered that this gene is expressed primarily in brain.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: neuro-degenerative diseases of the brain. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly neuro-degenerative diseases expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The predominant tissue distribution in the brain and homology to dynein, a microtubule motor protein involved in the positioning of cellular organelles and molecules suggests that the protein product of this clone would be useful for detection/treatment of neurodegenerative diseases, such as Alzheimers, Huntigtons, Parkinsons diseases and shizophrenia.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 372 as residues: Ser-89 to Thr-95.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:63 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3528 of SEQ ID NO:63, b is an integer of 15 to 3542, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 54

The translation product of this gene shares sequence homology with ubiquitin-conjugation protein, an enzyme which is thought to be important in the processing of the Huntingtons Disease causing gene.

It has been discovered that this gene is expressed primarily in brain and to a lesser extent in activated macrophages.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: neurodegenerative disease states including Huntingtons disease. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of brain tissues. For a number of disorders of the above tissues or cells, particularly of the neurological systems expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The predominant tissue distribution of this gene in the brain and its homology to a Huntington interacting protein suggests that the protein product of this clone

would be useful for the regulation of the expression of the Huntington disease gene and other neurodegenerative diseases including spinocerebellar ataxia types I and III, dentatorubropallidoluysian and spinal bulbar muscular atrophy. In addition, the existence of elevated levels of free ubiquitin pools in Alzheimers disease,

5 Parkinson's disease and amyotrophic lateral sclerosis indicates that the ubiquitin pathway of protein degradation plays a role in these disease states. Thus, considering the gene described here is homologous to a ubiquitin-conjugation protein it may play a general role in neurodegenerative conditions.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
10 373 as residues: Asn-19 to Arg-25, Arg-31 to Tyr-36, Glu-44 to Asp-52, Glu-57 to Gly-67, Leu-102 to Lys-108, Ser-135 to Gly-141.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:64 and may have been publicly available prior to conception of
15 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 869 of SEQ ID NO:64, b
20 is an integer of 15 to 883, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 56

25 The gene encoding the disclosed cDNA is believed to reside on chromosome 9. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 9.

It has been discovered that this gene is expressed primarily in T-cells (anergic T-cells, resting T-Cells, apoptotic T-cells) and lymph node (breast), as well as brain
30 (hypothalamus, hippocampus, pituitary, infant brain, early-stage brain).

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for

diagnosis of the following diseases and conditions: immune (e.g. immunodeficiencies, autoimmunities, inflammation, leukemias & lymphomas) and neurological (e.g. Alzheimer's disease, dementia, schizophrenia) disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous, hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful in the intervention or detection of pathologies associated with the hematopoietic and immune systems, such as anemias (leukemias). Expression of this gene product in immune cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. In addition, the expression in brain (including fetal) might suggest a role in developmental brain defects, neuro-degenerative diseases or behavioral abnormalities (e.g. schizophrenia, Alzheimer's, dementia, depression, etc.). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 375 as residues: Thr-48 to Ala-66.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:66 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 718 of SEQ ID NO:66, b is an integer of 15 to 732, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where the b is greater than or equal to a + 14.

15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 57**

It has been discovered that this gene is expressed primarily in lung, and to a lesser extent in a variety of other hematological cell types (e.g. Raji cells, bone marrow cell line, activated monocytes).

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: pulmonary and/or hematological disfunction. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vasculo-pulmonary and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful in the intervention and detection of pathologies associated with the vasculo-

pulmonary system. In addition the expression of this gene in a variety of leukocytic cell types and a bone marrow cell line might suggest a role in hematopoietic and immune system disorders, such as leukemias & lymphomas, inflammation, immunodeficiencies and autoimmunities.

5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 376 as residues: Met-1 to Glu-7, Arg-39 to Ser-55, Lys-59 to Glu-66, Leu-70 to Asn-77.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
10 related to SEQ ID NO:67 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
15 general formula of a-b, where a is any integer between 1 to 617 of SEQ ID NO:67, b is an integer of 15 to 631, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where the b is greater than or equal to a + 14.

20 **FEATURES OF PROTEIN ENCODED BY GENE NO: 58**

The translation product of this gene shares sequence homology with adenylate kinase isozyme 3 (gi|163528 GTP:AMP phosphotransferase (EC 2.7.4.10) [Bos taurus]), which is thought to be important in catalyzing the phosphorylation of AMP to ADP in the presence of ATP or inorganic triphosphate.

25 It has been discovered that this gene is expressed primarily in fetal liver, heart and placenta, and to a lesser extent in many other tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: hepatic, cardiovascular or
30 reproductive disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,

particularly of the hepatic, cardiovascular and reproductive systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the treatment and diagnosis of conditions related to hepatic function and pathogenesis, in particular, those dealing with liver development and the differentiation of hepatocyte progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 377 as residues: Gln-160 to Glu-167, Glu-177 to Pro-182, Ser-197 to Ile-204, Lys-215 to Ser-224.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:68 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1737 of SEQ ID NO:68, b is an integer of 15 to 1751, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 59

It has been discovered that this gene is expressed primarily in CD34 positive cells (Cord Blood).

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for

diagnosis of the following diseases and conditions: hematopoietic differentiation and immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful in the detection and treatment of conditions associated with CD34-positive cells, and therefore as a marker for cell differentiation in hematopoiesis, as well as immunological disorders. Expression of this gene product in hematopoietic cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 378 as residues: Lys-56 to Gly-71.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:69 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 494 of SEQ ID NO:69, b is an integer of 15 to 508, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 60

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 379 as residues: Asp-35 to Lys-49.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:70 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 231 of SEQ ID NO:70, b is an integer of 15 to 245, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 61

It has been discovered that this gene is expressed primarily in schizophrenic frontal cortex.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: nervous system and cognitive disorders. Similarly, polypeptides and antibodies directed to those polypeptides are

useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the frontal cortex and CNS expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, treatment and diagnosis of frontal cortex, neuro-degenerative and CNS disorders such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered bahaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 380 as residues: Thr-22 to Thr-27.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:71 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 347 of SEQ ID NO:71, b is an integer of 15 to 361, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where the b is greater than or equal to a + 14.

RES OF PROTEIN ENCODED BY GENE NO: 62

It has been discovered that this gene is expressed primarily in human adrenal nor, and to a lesser extent in human kidney medulla and adult pulmonary

Therefore, nucleic acids of the invention are useful as reagents for differential ation of the tissue(s) or cell type(s) present in a biological sample and for s of the following diseases and conditions: metabolic and endocrine

. Similarly, polypeptides and antibodies directed to those polypeptides are provide immunological probes for differential identification of the tissue(s) pe(s). For a number of disorders of the above tissues or cells, particularly of crine and nervous system disorders and neoplasia, expression of this gene at ntly higher or lower levels may be detected in certain tissues or cell types icerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, fluid or spinal fluid) taken from an individual having such a disorder, o the standard gene expression level, i.e., the expression level in healthy m an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be r study, treatment and diagnosis of neurological and endocrine disorders g neoplasia. Protein, as well as, antibodies directed against the protein may lity as a tumor marker and/or immunotherapy targets for the above listed

referred epitopes include those comprising a sequence shown in SEQ ID NO. sidues: Ile-20 to Leu-25.

Many polynucleotide sequences, such as EST sequences, are publicly and accessible through sequence databases. Some of these sequences are SEQ ID NO:72 and may have been publicly available prior to conception of nt invention. Preferably, such related polynucleotides are specifically from the scope of the present invention. To list every related sequence cumbersome. Accordingly, preferably excluded from the present invention r more polynucleotides comprising a nucleotide sequence described by the ormula of a-b, where a is any integer between 1 to 699 of SEQ ID NO:72, b

FEATURES OF PROTEIN ENCODED BY GENE NO: 62

It has been discovered that this gene is expressed primarily in human adrenal gland tumor, and to a lesser extent in human kidney medulla and adult pulmonary tissue.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: metabolic and endocrine disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous system disorders and neoplasia, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, treatment and diagnosis of neurological and endocrine disorders including neoplasia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 381 as residues: Ile-20 to Leu-25.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:72 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 699 of SEQ ID NO:72, b

is an integer of 15 to 713, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where the b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 63

It has been discovered that this gene is expressed primarily in human adipocytes, and to a lesser extent in spleen, 12-week old human, and testes.

Therefore, nucleic acids of the invention are useful as reagents for differential
10 identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immune, metabolic and growth disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of
15 the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

20 The tissue distribution suggests that the protein product of this clone would be useful for study, diagnosis and treatment of immune, developmental and metabolic disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:73 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
30 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 848 of SEQ ID NO:73, b is an integer of 15 to 862, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:73, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 64

5 One translated product of this clone is homologous to the mouse zinc finger protein PZF. (See Accession No. 453376; see also Gene 152 (2), 233-238 (1995).) Preferred polypeptide fragments correspond to the highly conserved domains shared between mouse and man. For example, preferred polypeptide fragments comprise the amino acid sequence:

10 LQCEICGFTCRQKASLNWHMKKHDADSFYQFSCNICGKKFEKKDSVVAHKA
KSHPEV (SEQ ID NO: 674), ITSTDILGTNPESLTQPSD (SEQ ID NO: 675),
NSTSGECLLLEAEGMSKSY (SEQ ID NO: 676),
CSGTERVSLMADGKIFVGSGSSG GTEGLVMNSDILGATTEVLIEDSDSAGP
(SEQ ID NO: 677),
15 IQYVRCMEGCGTVLAHPRYLQHHIKYQHLLKKKYVCPHPSCGRLFRLQKQL
LRHAKHHT (SEQ ID NO: 678),
DQRDYICEYCARAFKSSHNLAVHRMIHTGEK (SEQ ID NO: 679),
SALPQEVSIAASRPSRGWRSSRTSVSRHRDTENTRSSRSKTGSLQLICK
SEPNTDQLDY (SEQ ID NO: 680), PFKDDPRDETYKPHLERETPKPRRKSG
20 (SEQ ID NO: 681),
QYVRCMEGCGTVLAHPRYLQHHIKYQHLLKKKYVCPHPSCGRLFRL
QKQLLRHAKHHTD (SEQ ID NO: 682), and
QRDYICEYCARAFKSSHNLAVHRMI HTGEKHY (SEQ ID NO: 683). Also
preferred are polynucleotide fragments encoding these polypeptide fragments. When
25 tested against renal messangial cell lines, supernatants removed from cells containing
this gene induced a calcium flux in the renal cells tested in the FLIPR assay (small
molecule concentration and membrane permeability assay). Thus, it is likely that this
gene activates renal messangial cells via the binding of a ligand to a receptor. The
FLIPR assay indicates the binding of a ligand to a receptor, which is known to alter
30 intracellular levels of small molecules, such as calcium, potassium, sodium, and pH,
as well as alter membrane potential. Alterations in small molecule concentration can
be measured to identify supernatants which bind to receptors of a particular cell.

It has been discovered that this gene is expressed primarily in Rhabdomyosarcoma, Melanocyte and colon cancer tissue and to a lesser extent in smooth muscle, pancreatic tumor, and apoptotic T cells.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hemopoetic, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, diagnosis and treatment of cancer and hematopoietic disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 383 as residues: Phe-10 to Ile-46, Val-54 to Pro-91, Lys-123 to Pro-129, His-150 to Tyr-156, Thr-179 to Asn-185.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:74 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4588 of SEQ ID NO:74, b is an integer of 15 to 4602, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 65

It has been discovered that this gene is expressed primarily in human adipose and salivary gland tissue and to a lesser extent in human bone marrow and fetal
5 kidney.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: metabolic and immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to
10 provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the metabolic and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal
15 fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, diagnosis of metabolic and immune disorders. Protein, as well as,
20 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:75 and may have been publicly available prior to conception of
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1241 of SEQ ID NO:75, b
30 is an integer of 15 to 1255, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:75, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 66

This translated product of this gene was recently identified as oxytocinase splice variant 1. (See Accession Nos. 2209276 and d1010078.) Preferred polypeptide fragments comprise the amino acid sequence:

EMFDSL SYFKGSSLLLMLKTYLSEDVFQHAVV

LYLHNHSYASIQSDDLWDSFNEVTNQTL DVKRMMKTWTLQKGFPLVTVQK
KGKELFIQQERFFLNMKPEIQPSDTRYM (SEQ ID NO: 739). Also preferred are

polynucleotide fragments encoding this polypeptide fragment. The gene encoding the disclosed cDNA is believed to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

This gene is expressed primarily in tonsils, and to a lesser extent, in fetal liver spleen.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immune, hematopoietic, or developmental disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or hematopoietic system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g. immune, hematopoietic, developmental, hepatic, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution in fetal liver spleen and tonsils suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or

0982171-051801

leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 385 as residues: Pro-99 to Tyr-108.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:76 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 461 of SEQ ID NO:76, b is an integer of 15 to 475, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 67

It has been discovered that this gene is expressed primarily in hematopoietic cells, particularly apoptotic T-cells, and to lesser extent in primary dendritic cells and adipose tissue.

Therefore, nucleic acids of the invention are useful as reagents for differential
 5 identification of apoptotic T-cells, primary dendritic cells, and adipose tissue present in a biological sample and for diagnosis of the following diseases and conditions: hematopoietic diseases including cancer and general immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For
 10 a number of disorders of the above tissues or cells, particularly of the oral and intestinal mucosa as well as hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder,
 15 relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for treatment of diseases of the immune system, including cancer, hematopoietic and infectious diseases. Protein, as well as, antibodies directed against
 20 the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 386 as residues: Ala-2 to Ala-9, Ser-36 to Trp-41.

Many polynucleotide sequences, such as EST sequences, are publicly
 25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:77 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
 30 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 451 of SEQ ID NO:77, b is an integer of 15 to 465, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:77, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 68

5 It has been discovered that this gene is expressed primarily in kidney cortex and to a lesser extent in infant brain, heart, uterus, and blood.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of kidney tissue present in a biological sample and for diagnosis of the following diseases and conditions: soft tissue cancer, inflammation, kidney fibrosis.

10 Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and endocrines systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and
15 wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be
20 useful for study and treatment of cancer and fibroses. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 387 as residues: Arg-8 to Lys-20, Asp-28 to Glu-33, Val-42 to Thr-47, Glu-52 to Ser-
25 57, Thr-65 to Phe-73, Gly-75 to Tyr-85, Ala-90 to Arg-95, Arg-111 to Gln-124, Lys-134 to Ser-140, Phe-165 to Asp-170, Pro-199 to Cys-205, Gly-239 to Val-244, Glu-247 to Trp-253, Tyr-319 to Gln-326, Leu-423 to Asn-433.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
30 related to SEQ ID NO:78 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

"SEQUENCE" 1.0513001

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1893 of SEQ-ID NO:78, b is an integer of 15 to 1907, where both a and b correspond to the positions of
 5 nucleotide residues shown in SEQ ID NO:78, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 69

The translation product of this gene shares strong sequence homology with
 10 vertebrate and invertebrate protein tyrosine phosphatases. The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

It has been discovered that this gene is expressed primarily in endometrial tumors, melanocytes, myeloid progenitors and to a lesser extent in infant brain,
 15 adipocytes, and several hematopoietic stem cells. Therefore, nucleic acids of the invention are useful as reagents for differential identification of transformed hematopoietic and epithelial cells present in a biological sample and for diagnosis of the following diseases and conditions: cancer of skin and endometrium, leukemia. Similarly, polypeptides and antibodies directed to those polypeptides are useful to
 20 provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal
 25 fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and sequence similarity with tyrosine phosphatases suggests that the protein product of this clone would be useful for study and treatment
 30 of cancer and hematopoietic disorders. Expression within embryonic tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division. Additionally, the expression in

09882171.061601

hematopoietic cells and tissues suggests that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 388 as residues: Met-1 to Gly-6.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:79 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1154 of SEQ ID NO:79, b is an integer of 15 to 1168, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 70

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Thus, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

It has been discovered that this gene is expressed primarily in osteoclastoma, breast, and infant brain and to a lesser extent in various fetal and transformed bone, ovarian, and neuronal cells.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: degenerative conditions of the brain and skeleton. Similarly, polypeptides and antibodies directed to those

5 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and skeletal system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or

10 spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study and treatment of degenerative, neurological and skeletal disorders.

15 Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:80 and may have been publicly available prior to conception of

20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1271 of SEQ ID NO:80, b

25 is an integer of 15 to 1285, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 71

30 This gene was originally cloned from tumor cell lines. Recently another group has also cloned this gene, calling it the human malignant melanoma metastasis-suppressor (KiSS-1) gene. (See Accession No. U43527). Preferred polypeptide

fragments comprise the amino acid sequence: LEKVASVGNSRPTGQQLESGLLA (SEQ ID NO: 685), VHREEASCYCQAEPGDL (SEQ ID NO: 686), RPALRQAGGGTREPRQKRWAGL (SEQ ID NO: 687), and AVNFRPQRSQSM (SEQ ID NO: 688). Any frame shifts can easily be resolved using known molecular biology techniques. The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in many types of carcinomas and to a lesser extent in many normal organs.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissues(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer, particularly melanomas, and other hyperproliferative disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of transformed organ tissue, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder. As a tumor suppressor gene, increase amounts of the polypeptide can be used to treat patients having a particular cancer.

The tissue distribution indicates that this gene and the translated product is useful for the diagnosis and study of cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 390 as residues: Gly-29 to Leu-38, Pro-52 to Ala-59, Ser-65 to Ser-70, Pro-73 to Gly-85, Pro-89 to Pro-95, Arg-105 to Trp-114, Pro-128 to Arg-137.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:81 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:81, b is an integer of 15 to 1290, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 72

It has been discovered that this gene is expressed primarily in striatum and to a lesser extent in adipocytes and hemangiopericytoma.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of striatal cells present in a biological sample and for diagnosis of the following diseases and conditions: neurological disorders as well as fat and lysosomal storage diseases. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis, study and treatment of neurodegenerative and growth disorders, such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, and schizophrenia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:82 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 670 of SEQ ID NO:82, b is an integer of 15 to 684, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:82, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 73

It has been discovered that this gene is expressed primarily in bone marrow stromal cells and to a lesser extent in smooth muscle, testes, endothelium, and brain.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of bone marrow present in a biological sample and for diagnosis of the following diseases and conditions: connective tissue and hematopoietic diseases. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, diagnosis, and treatment of connective tissue and blood diseases.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 392 as residues: Thr-17 to Glu-24, Gly-28 to Pro-45, Ser-47 to Pro-59, Lys-62 to Asp-79, Gly-91 to Gly-99, Ser-144 to Leu-157, Gln-199 to Thr-210, Thr-215 to Ser-221, Pro-231 to Ser-247.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:83 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2010 of SEQ ID NO:83, b is an integer of 15 to 2024, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:83, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 74

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

It has been discovered that this gene is expressed primarily in brain, fetal liver and lung and to a lesser extent in retina, spinal chord, activated T-cells and endothelial cells.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of brain and regenerating liver present in a biological sample and for diagnosis of the following diseases and conditions: CNS and spinal chord injuries, immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine,

synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study and treatment of hematopoietic disorders, such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia. Furthermore, the tissue distribution also suggests that the protein product of this clone would be useful for the study and treatment of neurological disorders such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, and schizophrenia.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 393 as residues: Pro-55 to Lys-63.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:84 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 917 of SEQ ID NO:84, b is an integer of 15 to 931, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:84, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 75

The translation product of this gene shares sequence homology with GTP binding proteins (intracellular).

It has been discovered that this gene is expressed primarily in bone marrow, brain, and melanocytes and to a lesser extent in various endocrine and hematopoietic tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: hematopietic and nervous system

09882474.051801

conditions. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and immune, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to nucleotide binding factors suggests that the protein product of this clone would be useful for study, diagnosis, and treatment of brain degenerative, skin and blood diseases. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 394 as residues: Ala-3 to Glu-12, Glu-36 to Thr-41, Val-49 to Leu-60.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:85 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 811 of SEQ ID NO:85, b is an integer of 15 to 825, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:85, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 76

It has been discovered that this gene is expressed primarily in activated T-cells and to a lesser extent in retina, brain, and fetal bone.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of activated T-cells and developing brain present in a biological sample and for diagnosis of the following diseases and conditions: immune deficiencies and skeletal and neuronal growth disorders. Similarly, polypeptides and antibodies
 5 directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous, immune, and skeletomuscular systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or
 10 bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis, study and treatment of cancer, urogenital, and brain degenerative
 15 diseases. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:86 and may have been publicly available prior to conception of
 20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1224 of SEQ ID NO:86, b
 25 is an integer of 15 to 1238, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:86, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 77

30 It has been discovered that this gene is expressed primarily in fetal liver, activated monocytes, osteoblasts and to a lesser extent in synovial, brain, and lymphoid tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of myeloid and lymphoid present in a biological sample and for diagnosis of the following diseases and conditions: inflammation, immune deficiencies, cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and skeleton, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for study, diagnosis, and treatment of lymphoid and mesenchymal cancers and nervous system diseases. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 396 as residues: Asp-129 to Ser-134, Arg-159 to Ala-168, Arg-258 to Pro-264.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:87 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1446 of SEQ ID NO:87, b is an integer of 15 to 1460, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:87, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 78

The translation product of this gene shares sequence homology with polymerase polyprotein precursor which is thought to be important in DNA repair and replication

It has been discovered that this gene is expressed primarily in infant brain and to a lesser extent in tumors and tumor cell lines

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer, especially of the neural system and developing organs. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural system expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to polymerase polyprotein precursor suggests that the protein product of this clone would be useful for diagnosis and treatment of cancers, especially of the neural system and developing organs. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:88 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1381 of SEQ ID NO:88, b is an integer of 15 to 1395, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:88, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 79

5 It has been discovered that this gene is expressed primarily in muscle and endothelial cells and to a lesser extent in brain.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: vascular diseases. Similarly,
10 polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids
15 (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for treatment and diagnosis of disorders of the vascular and neural system
20 including cardiovascular and endothelial. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 398 as residues: Tyr-5 to Glu-13.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:89 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
30 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1172 of SEQ ID NO:89, b

is an integer of 15 to 1186, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:89, and where the b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 80

This gene appears to be a human homolog of a mouse metalloproteinase/disintegrin protein, which is thought to play a role in skeletal muscle development involving the formation of multi-nucleated myotubes. The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis of chromosome 10.

It has been discovered that this gene is expressed primarily in placenta and to a lesser extent in fetal liver

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: developmental disorders and disorder of the haemopoietic system, fetal liver and placenta. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of developmental disorders and disorder of the hematopoietic system, fetal liver and placenta, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis and treatment of developmental disorders and disorders of the hematopoietic system, fetal liver and placenta. Alternatively, the tissue distribution and nucleotide homology to metalloproteinase/disintegrin protein suggest that the translation product of this gene may be important in the fetal development of skeletal

muscle. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 399 as residues: Cys-34 to Tyr-41, Lys-53 to Lys-68.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:90 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1807 of SEQ ID NO:90, b is an integer of 15 to 1821, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:90, and where the b is greater than or equal
15 to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 81

It has been discovered that this gene is expressed primarily in bone marrow, placenta and tissues and organs of the hematopoietic system

20 Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: disorders of the bone and hematopoietic system. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification
25 of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, bone and hematopoietic system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder,
30 relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

098271.061801
T09T90 T22860

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis and treatment of disorders of the immune, bone and hematopoietic system, such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:91 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 848 of SEQ ID NO:91, b is an integer of 15 to 862, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:91, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 82

The translation product of this gene shares sequence homology with secretory carrier membrane protein which is thought to be important in protein transport and export. Any frame shifts in coding sequence can be easily resolved using standard molecular biology techniques. Another group recently cloned this gene, calling it SCAMP. (See Accession No. 2232243.). The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, the polynucleotides related to this invention are useful as a marker in linkage analysis of chromosome 1.

It has been discovered that this gene is expressed primarily in prostate, breast and spleen, and to a lesser extent in several other tissues and organs

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: disorders of the breast prostate and spleen. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s)

or cell type(s). For a number of disorders of the above tissues or cells, particularly disorders of the breast prostate and spleen, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to secretory carrier membrane protein suggests that the protein product of this clone would be useful for diagnosis and treatment of disorders of the breast, prostate and spleen. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 401 as residues: Ala-2 to Glu-13, Ser-15 to Gln-22, Pro-43 to Ala-57, Gln-70 to Gln-89, Leu-100 to Ser-119.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:92 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 682 of SEQ ID NO:92, b is an integer of 15 to 696, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:92, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 83

It has been discovered that this gene is expressed primarily in developing organs and tissue like placenta and infant brain and to a lesser extent in developed organs and tissue like cerebellum and heart

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: neurological diseases. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for treatment and diagnosis of diseases of the neural system including neurological disorders and cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:93 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1872 of SEQ ID NO:93, b is an integer of 15 to 1886, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:93, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 84

The translation product of this gene shares sequence homology with ATPase 6 in *Trypanosoma brucei* which is thought to be important in metabolism.

It has been discovered that this gene is expressed primarily in tumor and fetal tissues and to a lesser extent in melanocytes, kidney cortex, monocytes and ovary.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
5 diagnosis of the following diseases and conditions: metabolism disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal systems, expression of this gene at significantly higher or lower levels may be detected in
10 certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to ATPase indicates that the protein
15 product of this clone would be useful for treatment and diagnosis of metabolism disorders, especially in fetal and tumor tissue growth. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
20 403 as residues: Gln-17 to Ile-22, Gln-54 to Ser-60.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:94 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
25 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1760 of SEQ ID NO:94, b is an integer of 15 to 1774, where both a and b correspond to the positions of
30 nucleotide residues shown in SEQ ID NO:94, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 85

The translation product of this gene shares sequence homology with the immunoglobulin superfamily of proteins which are known to be important in immune response and immunity.

5 It has been discovered that this gene is expressed primarily in stromal cells, colon cancer, lung, amygdala, melanocyte and to a lesser extent in a variety of other tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
10 diagnosis of the following diseases and conditions: defects of stromal cell development and cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the stromal cells, expression of this gene at significantly higher or
15 lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

20 The tissue distribution and homology to immunoglobulin indicates that the protein product of this clone would be useful for treatment and diagnosis of immune system disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:95 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
30 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1765 of SEQ ID NO:95, b is an integer of 15 to 1779, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:95, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 86

The translation product of this gene shares sequence homology with transcription initiation factor eIF-4 gamma which is thought to be important in gene transcription. Additionally, the translation product of this gene shares significant homology to a Homo sapiens polyadenylate binding protein-interacting protein-1 (PAIP1), which could play a possible role as an RNA editing enzyme or polypeptide, the defect of which could lead to translational errors or cancer. One embodiment of this clone comprises polypeptides of the following amino acid sequence:

HQPPQPKAPGF (SEQ ID NO: 689),

GAQCEVPASPQRPSRPGALPEQTRPLRAPPSSQDKIPQQNSESAMAKPQVVVA
PVLMSKLSVNAPEF (SEQ ID NO: 690),

EDGCEDYPTLSEYVQDFLNHLTEQPGSFETEI

EQFAETLNGCVTTDDALQELVELIYQQATSIPNFSYMGARLCNYLSHHLTISP
QSGNFRQLLLQRCRTEYEVKDQAAKGDEVTRKRFHAFVLFLGELYLNLEIKG
TNGQVTRADILQVGLRELLNALFSNPMDDNLICAVKLLKLTGSVLEDAWKEK
GKMDEEIIQRIENVVLDANCSRVDKQMLLKLVELRSSNWGRVHATSTYRE

ATPENDPNYFMNEPTFYTSDGVPFTAADPDYQEKYQELLEREDFFPDYEENG
TDLSGAGDPYL (SEQ ID NO: 691), or AYEKFCLESERKRKQ (SEQ ID NO:

692). Recently another group published a paper in which they described a Homo sapiens polyadenylate binding protein-interacting protein-1 (PAIP1) mRNA, including the complete coding sequence (Genbank accession AF013758, Nature 392,

520-523 (1998)).

It has been discovered that this gene is expressed primarily in tumor tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: tumorigenesis. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly in tumor tissues,

expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to transcription initiation factor eIF-4 gamma and Homo sapiens polyadenylate binding protein-interacting protein-1 (PAIP1) suggests that the protein product of this clone would be useful for gene regulation in tumorigenesis. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 405 as residues: Met-1 to Arg-15.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:96 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2787 of SEQ ID NO:96, b is an integer of 15 to 2801, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:96, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 87

The translation product of this gene shares sequence homology at low level in prolines with secreted basic proline-rich peptide II-2 which is thought to be important in protein structure or inhibiting hydroxyapatite formation in vitro.

It has been discovered that this gene is expressed primarily in endometrial tumor and fetal lung.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: endometrial tumors. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the muscular/skeletal and reproductive systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to secreted basic proline-rich peptide II-2 suggests that the protein product of this clone would be useful for inhibiting hydroxyapatite formation or establishing cell/tissue structure. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 406 as residues: Glu-175 to Glu-193.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:97 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1617 of SEQ ID NO:97, b is an integer of 15 to 1631, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:97, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 88

It has been discovered that this gene is expressed primarily in: amniotic cells induced with TNF in culture; and to a lesser extent in colon tissue from a patient with Crohn's Disease; parathyroid tumor; activated T-cells; cells of the human Caco-2 cell line; adenocarcinoma; colon; corpus colosum; fetal kidney; pancreas tumor; fetal
5 brain; early stage brain, and anergic T-cells.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: tumors. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological
10 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system; e.g., tumors, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual
15 having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for modulating tumorigenesis and other immune system conditions such as disorders in immune response. Protein, as well as, antibodies directed against the protein may
20 show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 407 as residues: Pro-61 to Glu-75.

Many polynucleotide sequences, such as EST sequences, are publicly
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:98 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
30 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 490 of SEQ ID NO:98, b is an integer of 15 to 504, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:98, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 89

5 The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis of chromosome 1.

It has been discovered that this gene is expressed primarily in fetal liver/spleen and hematopoietic cells and to a lesser extent in brain, osteosarcoma, and testis tumor.

10 Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: leukemia and hematopoietic disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s)
15 or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene
20 expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis and treatment of hematopoietic and immune disorders.

Expression of this gene product in hematopoietic cells suggests a role in the
25 regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of
30 lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid

09882171.061801

arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 408 as residues: Gly-13 to Cys-18, Arg-30 to Ser-36, Ala-53 to Phe-58.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:99 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1402 of SEQ ID NO:99, b is an integer of 15 to 1416, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:99, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 90

The translation product of this gene shares weak sequence homology with mouse Gcap1 protein which is developmentally regulated in brain. The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides of this invention are useful as a marker in linkage analysis for chromosome 11.

It has been discovered that this gene is expressed primarily in infant and adult brain and fetal liver/spleen and to a lesser extent in smooth muscle, T cells, and a variety of other tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: neurological or hematopoietic

disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous, hematopoietic, immune, and endocrine systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and its homology to Gcap1 protein suggests that the protein product of this clone would be useful for treating and diagnosis of disorders in neuronal, hematopoietic, immune, and endocrine systems. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:100 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2833 of SEQ ID NO:100, b is an integer of 15 to 2847, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:100, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 91

It has been discovered that this gene is expressed primarily in brain and hematopoietic cells and to a lesser extent in tumor tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: disorder in nervous,

hematopoietic, immune systems and tumorigenesis. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the in nervous, hematopoietic, immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of disorders in the nervous, hematopoietic, and immune systems. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:101 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1380 of SEQ ID NO:101, b is an integer of 15 to 1394, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:101, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 92

The translation product of this gene shares sequence homology with neuroendocrine-specific protein A which is thought to be important in neurologic systems.

It has been discovered that this gene is expressed primarily in brain tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for

diagnosis of the following diseases and conditions: neural disorders and degeneration disease. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central or peripheral nervous systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to neuroendocrine-specific protein A suggests that the protein product of this clone would be useful for treatment or diagnosis of neural disorders and degeneration disease. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 411 as residues: Glu-65 to Gln-70.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:102 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 780 of SEQ ID NO:102, b is an integer of 15 to 794, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:102, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 93

The translation product of this gene shares sequence homology with collagen-like protein and prolin-rich protein which are thought to be important in connective tissue function and tissue structure.

It has been discovered that this gene is expressed primarily in fetal liver/spleen and brain tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: neuronal or hematopoietic disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and hematopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder:

The tissue distribution and homology to collagen-like protein and proline-rich proteins suggests that the protein product of this clone would be useful for supporting brain and hematopoietic tissue function and diagnosis and treatment of disorders in these functions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:103 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1530 of SEQ ID NO:103, b is an integer of 15 to 1544, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:103, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 94

5 It has been discovered that this gene is expressed primarily in embryonic tissues and tumor tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system (e.g., tumors), expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis and treatment of cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 413 as residues: Pro-39 to Leu-46, Pro-96 to Arg-103, Pro-117 to Ser-124.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:104 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 857 of SEQ ID NO:104, b is an integer of 15 to 871, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:104, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 95

It has been discovered that this gene is expressed primarily in brain tumor, placenta, and melanoma.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: brain tumor or melanoma.

Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain or melanocytes, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the translation product of this gene is useful in the diagnosis and treatment of brain tumors and melanoma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 414 as residues: Ser-44 to Glu-50, Pro-53 to Gly-60.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:105 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 390 of SEQ ID NO:105, b is an integer of 15 to 404, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:105, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 96

5 The translation product of this gene shares sequence homology with a yeast membrane protein, SUR4, which encodes for APA1 that acts on a glucose-signaling pathway that controls the expression of several genes that are transcriptionally regulated by glucose. The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a
10 marker in linkage analysis for chromosome 1.

It has been discovered that this gene is expressed primarily in fetal liver, and to a lesser extent in placenta and breast tissue.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
15 diagnosis of the following diseases and conditions: defects of fetal liver or defects of glucose-regulated ATPase activities in tissues. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal immune/hematopoietic system,
20 expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

25 The tissue distribution and homology to yeast SUR4 membrane protein suggests that the protein product of this clone would be useful for diagnosis and treatment of defects of fetal liver or defects of glucose-regulated ATPase activities. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 415 as residues: Ala-15 to Gln-20, Tyr-89 to Glu-103, His-253 to Leu-261.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:106 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1528 of SEQ ID NO:106, b is an integer of 15 to 1542, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:106, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 97

The gene encoding the disclosed cDNA is thought to reside on chromosome 18. Accordingly, polynucleotides related to the invention are useful as a marker in linkage analysis for chromosome 18.

It has been discovered that this gene is expressed primarily in fetal liver, brain, and amniotic fluid.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: defects of the fetal immune system and adult brain. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal immune system and adult brain, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for detecting defects of the fetal immune and hematopoietic systems since fetal liver

is the predominant organ responsible for hematopoiesis in the fetus. In addition, the gene product of this gene is thought to be useful for detecting certain neurological defects of the brain. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:107 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2313 of SEQ ID NO:107, b is an integer of 15 to 2327, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:107, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 98

The translation product of this gene shares sequence homology with an yolk protein precursor, Vitellogenin which is thought to be important in binding lipids such as phosvitin. The gene encoding the disclosed cDNA is thought to reside on chromosome 10. Accordingly, polynucleotides related to the invention are useful as a marker in linkage analysis for chromosome 10.

It has been discovered that this gene is expressed primarily in amnionic cells and fetal liver.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: defects in amnionic cells, fetal liver development and the fetal immune system. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal liver and developing tissues,

expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the
 5 expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to vitellogenin indicates that the protein product of this clone is useful for treatment and diagnosis of defects in amnionic cells, fetal liver development and the fetal immune system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or
 10 immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 417 as residues: Pro-24 to Ala-32.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
 15 related to SEQ ID NO:108 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
 20 general formula of a-b, where a is any integer between 1 to 1048 of SEQ ID NO:108, b is an integer of 15 to 1062, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where the b is greater than or equal to a + 14.

25 **FEATURES OF PROTEIN ENCODED BY GENE NO: 99**

It has been discovered that this gene is expressed primarily in placenta, endometrial tumor, osteosarcoma and stromal cells.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
 30 diagnosis of the following diseases and conditions: tumor of the endometrium or bone, and osteosarcoma. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification

of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the obsteric system (i.g. placenta, endometrium) and the bones, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for diagnosis and treatment of tumors and abnormalities of the endometrium, and the bones because of its abundance in the aforementioned tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 418 as residues: Leu-3 to Arg-8, His-11 to Glu-16, Leu-19 to Glu-27, Lys-67 to Glu-73, Tyr-79 to Asp-87, Lys-101 to Ile-107, Val-143 to Leu-155, Thr-162 to Ser-169.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:109 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2525 of SEQ ID NO:109, b is an integer of 15 to 2539, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:109, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 100

It has been discovered that this gene is expressed primarily in hepatocellular tumor.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for

diagnosis of the following diseases and conditions: hepatocellular tumor. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of hepatocellular cancer because of its abundant expression in this tissue. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 419 as residues: Ala-100 to Ser-109, His-138 to His-145, Glu-171 to Ser-182.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:110 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1737 of SEQ ID NO:110, b is an integer of 15 to 1751, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:110, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 101

The gene encoding the disclosed cDNA is thought to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

It has been discovered that this gene is expressed primarily in Corpus Colosum, fetal lung and infant brain.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: defects of the Corpus Colosum or defects of the fetal lung. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the Corpus Colosum and brain in general, and fetal lung, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of defects of the Corpus Colosum and brain in general, and defects of fetal lung. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:111 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1103 of SEQ ID NO:111, b is an integer of 15 to 1117, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:111, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 102

It has been discovered that this gene is expressed primarily in T cells and stromal cells, and to a lesser extent in adrenal gland.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
 5 diagnosis of the following diseases and conditions: defects of T cell immunity and stromal cell development. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or
 10 lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

15 The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of defects of T cell immunity and stromal cell development because of its abundant expression in these tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 421 as residues: Tyr-12 to Glu-17.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:112 and may have been publicly available prior to conception
 25 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1299 of SEQ ID NO:112,
 30 b is an integer of 15 to 1313, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:112, and where the b is greater than or equal to a + 14.

T08T90.T.051801

FEATURES OF PROTEIN ENCODED BY GENE NO: 103

It has been discovered that this gene is expressed primarily in infant brain and placenta.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: defects of the brain and nervous system. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, especially brain, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for detecting defects of the brain, especially in young children. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:113 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1640 of SEQ ID NO:113, b is an integer of 15 to 1654, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 105

It has been discovered that this gene is expressed primarily in human osteoclastoma and to a lesser extent in human pancreas tumor.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer, particularly osteoclastoma and pancreatic tumor. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly in transformed tissues, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of some types of tumors, particularly pancreatic cancer and osteoclastoma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 424 as residues: Glu-17 to Leu-23, Ala-148 to Leu-173.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:115 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 828 of SEQ ID NO:115, b is an integer of 15 to 842, where both a and b correspond to the positions of

09882171.061801
T08T90.TZ2886

nucleotide residues shown in SEQ ID NO:115, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 106

5 The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

 It has been discovered that this gene is expressed primarily in fetal liver/spleen, and to a lesser extent in activated T-Cells, 8 hrs.

10 Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For
15 a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the
20 expression level in healthy tissue from an individual not having the disorder.

 The tissue distribution suggests that the protein product of this clone would be useful for diagnosis or treatment of immune disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

25 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 425 as residues: Leu-31 to Lys-37.

 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:116 and may have been publicly available prior to conception
30 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

09832171.061801

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1626 of SEQ ID NO:116, b is an integer of 15 to 1640, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:116, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 107

It has been discovered that this gene is expressed primarily in human embryo and to a lesser extent in spleen and chronic lymphocytic leukemia.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: leukemia. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or hemopoietic systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for the diagnosis and treatment of leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 426 as residues: Gly-26 to Asn-31, Glu-53 to Gly-62.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:117 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

09882171.061801

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 938 of SEQ ID NO:117, b is an integer of 15 to 952, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:117, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 108

The gene encoding the disclosed cDNA is thought to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

It has been discovered that this gene is expressed primarily in placenta, and to a lesser extent in early stage human brain and in lung.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: fetal developmental abnormalities. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly in fetal and amniotic tissue, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this is useful for production of growth factor(s) associated with fetal development. Preferred polypeptides comprise the full-length polypeptide shown in the sequence listing, truncated however, at the amino terminus and beginning with QTIE. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 427 as residues: Pro-12 to Gly-22, Ile-57 to Cys-63, Leu-87 to Met-96, Ala-109 to Gln-118, Glu-144 to Phe-150.

09882171.061801
FOI 90-122886

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:118 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1242 of SEQ ID NO:118, b is an integer of 15 to 1256, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:118, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 109

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

It has been discovered that this gene is expressed primarily in fetal spleen, and to a lesser extent in B-Cell lymphoma and T-Cell lymphoma.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: lymphoma. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for the treatment and diagnosis of human lymphomas. Protein, as well as, antibodies

directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 428 as residues: Glu-9 to Arg-15, Pro-71 to Lys-79.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:119 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1129 of SEQ ID NO:119, b is an integer of 15 to 1143, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:119, and where the b is greater than or
15 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 110

The translation product of this gene shares sequence homology with sarcoma amplified sequence (SAS), a tetraspan receptor which is thought to be important in
20 malignant fibrous histiocyoma and liposarcoma. The translation product of this clone also shares sequence homology with Transmembrane 4 superfamily proteins. The transmembrane 4 superfamily (TM4SF), or tetraspan superfamily, is the second biggestsubfamily among CD antigen superfamilies. Members of this family appear to serve a role in the activation of T- cells, for example as an activation antigen of T-
25 cells. All TM4SF members contain four putative transmembrane domains, two extracellular loops, and two short cytoplasmic tails. One embodiment of this clone comprises polypeptides of the following amino acid sequence:

GPGPPPTSALLPRLGXAPKARTKQLSGNLRRS

RIYVQLPATTGSKMVCGGFACSKNCLCALNLLYTLVSLLLIGIAAWGIGFGLIS

30 SLRVVGVVIAVGIFLFLIALVGLIGAVKHHQVLLFFY (SEQ ID NO: 693). An additional embodiment would be the polynucleotides encoding these polypeptides.

"061801" 1771228860

It has been discovered that this gene is expressed primarily in human osteoclastoma, and to a lesser extent in pineal gland and infant brain.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
 5 diagnosis of the following diseases and conditions: malignant fibrous histiocytoma and liposarcoma. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or
 10 lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

15 The tissue distribution and homology to sarcoma amplified sequence (SAS) indicates that the protein product of this clone is useful for treatment of, osteosarcoma, malignant fibrous histiocytoma and liposarcoma and related cancers, particularly sarcomas. Alternatively, the homology to TM4SF proteins indicates that the translation product of this clone may function as an activating agent of T-cells and
 20 their immune response in the body. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 429 as residues: Asn-100 to Gly-105, Asn-114 to Leu-126, Ser-133 to Thr-139, Lys-
 25 146 to Cys-151, Tyr-188 to Pro-200.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:120 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
 30 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

09882171.051801

general formula of a-b, where a is any integer between 1 to 1768 of SEQ ID NO:120, b is an integer of 15 to 1782, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:120, and where the b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 111

The translation product of this gene shares sequence homology with 6.8K proteolipid protein, mitochondrial - bovine.

10 It has been discovered that this gene is expressed primarily in wilm's tumor and to a lesser extent in cerebellum and placenta.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: wilm's tumor. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide
15 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or renal systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an
20 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to 6.8K proteolipid protein indicates that the protein product of this clone is useful for diagnostic and therapeutics associated with tumors, particularly wilm's tumor disease. Protein, as well as, antibodies directed
25 against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 430 as residues: Asp-42 to Ala-47.

Many polynucleotide sequences, such as EST sequences, are publicly
30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:121 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

05832171.051801
108190"1228860

excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 596 of SEQ ID NO:121, b
 5 is an integer of 15 to 610, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:121, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 112

10 It has been discovered that this gene is expressed primarily in embryonic tissue and to a lesser extent in osteoblasts, endothelial cells, macrophages (GM-CSF treated), and bone marrow.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for
 15 diagnosis of the following diseases and conditions: immune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be detected in
 20 certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be
 25 useful for treatment or diagnosis of immune disorders. Expression of this gene product in immune cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a
 30 usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of

"061801" 71 22850

lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues. Preferred polypeptides encoded by this gene comprise the following amino acid sequence:

MITDVQLAIFANMLGVSLFLLVVLVLYHYVAVNNPKKQE (SEQ ID NO: 694).

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 431 as residues: Asn-20 to Glu-25.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:122 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 512 of SEQ ID NO:122, b is an integer of 15 to 526, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:122, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 113

The gene encoding the disclosed cDNA is thought to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

It has been discovered that this gene is expressed primarily in hepatocellular tumor, and to a lesser extent in fetal liver/spleen.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: tumors, particularly hepatocellular tumors. Similarly, polypeptides and antibodies directed to those polypeptides are
 5 useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hepatic system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an
 10 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of tumors, particularly hepatocellular tumors. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker
 15 and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:123 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
 20 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2067 of SEQ ID NO:123, b is an integer of 15 to 2081, where both a and b correspond to the positions of
 25 nucleotide residues shown in SEQ ID NO:123, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 114

The translation product of this gene exhibits a very high degree of sequence
 30 identity with the human Pig8 gene which is thought to be important in p53 mediated apoptosis. The sequence of this gene has since been published by Polyak and colleagues (Nature 389, 300-306 (1997)). In addition, the predicted translation

09882171.061801

product of this contig exhibits very high sequence homology with a murine gene denoted as EI24 which is also thought to be important in p53 mediated apoptosis.

It has been discovered that this gene is expressed primarily in infant brain and activated T-cells and to a lesser extent in bone marrow, fetal liver, and prostate.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer and tissue damage by radiation and anti-cancer drugs. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to human Pig8 and murine EI24 genes suggests that the protein product of this clone would be useful for preventing apoptosis in patients being treated with anti-oncogenic drugs such as etoposide, hydroperoxycyclophosphamide, and X-irradiation, since this protein product is upregulated in cells undergoing such treatment where p53 was overexpressed. It may also be useful in the treatment of hematopoietic disorders and in boosting numbers of hematopoietic stem cells by interfering with the apoptosis of progenitor cells. The mature polypeptide is predicted to comprise the following amino acid sequence:

EEMADSVKTFLQDLARGIKD
 SIWGICTISKLDARIQQKREEQRRRRASSVLAQRRRAQSIERKQSEPRIVSRIFQ
 CCAWNGGVFWFSLLLFYRVFIPVLQSVTARIIGDPSLHGDVWSWLEFFLTSIFS
 ALWVLPLFVLSKVVNAIWFQDIADLAFEVSGRKPHFPSPVSKIIADMLFNLLL
 QALFLIQGMFVSLFPIHLVGQLVSLHMSLLYSLYCFEYRWFNKGIEMHQRLS
 NIERNWPYYFGFGLPLAFLTAMQSSYIISGCLFSILFPLFIISANEAKTPGKAYLF
 QLRLFSLVVFLSNRLFHKTVYQLQSALSSSTSAEKFPSPHPSPAKLKATAGH
 (SEQ ID NO: 695). Accordingly, polypeptides comprising the foregoing amino acid

sequence are provided as are polynucleotides encoded such polypeptides. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 433 as residues: Asn-24 to Gly-30, Thr-65 to Ala-78.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:124 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1703 of SEQ ID NO:124, b is an integer of 15 to 1717, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:124, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 115

It has been discovered that this gene is expressed primarily in stromal cells and to a lesser extent in multiple sclerosis.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: affecting the nervous system. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for treatment and diagnosis of multiple sclerosis and other autoimmune diseases. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 434 as residues: Pro-28 to Ile-33, Lys-88 to Ser-93, Glu-182 to Pro-192.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:125 and may have been publicly available prior to conception
10 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 790 of SEQ ID NO:125, b
15 is an integer of 15 to 804, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:125, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 116

20 It has been discovered that this gene is expressed primarily in the gall bladder.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: gall stones or infection of the digestive system . Similarly, polypeptides and antibodies directed to those
25 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system or renal system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine,
30 synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for possible prevention of digestive disorders where there may be a lack of digestive enzymes produced or in the detection and possible prevention of gall stones. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 435 as residues: Lys-32 to Val-37.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:126 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 417 of SEQ ID NO:126, b is an integer of 15 to 431, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:126, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 117

The translation product of this gene shares sequence homology with the dystrophin gene which is thought to be important in the building and maintainance of muscles. The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

It has been discovered that this gene is expressed primarily in placenta and to a lesser extent in fetal brain and fetal liver spleen.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: muscular dystrophy, Duchenne and Becker's muscular dystrophies. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification

of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal muscle system, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to the dystrophin gene suggests that the protein product of this clone would be useful for diseases related the degenerative myopathies that are characterized by the weakness and atrophy of muscles without neural degradation; such as Duchenne and Becker's muscular dystrophies. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 436 as residues: Lys-2 to Val-9, Lys-52 to Leu-58, Gln-88 to Asp-99, Met-115 to Val-122, Arg-124 to Glu-135, Glu-143 to Pro-159, Ser-167 to Ile-174, Glu-190 to Leu-195, Arg-237 to Arg-248, Asp-275 to Tyr-281, Pro-293 to Glu-308, Ile-329 to Arg-335, Gln-341 to Gln-347, Arg-355 to Trp-362.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:127 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3738 of SEQ ID NO:127, b is an integer of 15 to 3752, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:127, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 118

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

5 It has been discovered that this gene is expressed primarily in olfactory and to a lesser extent in cartilage.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: connective tissue diseases; chondrosarcomas. Similarly, polypeptides and antibodies directed to those
10 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the connective tissue, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from
15 an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and suggests that the protein product of this clone would be useful for tumors of connective tissues, osteoarthritis and the treatment and diagnosis of chondrosarcoma. Protein, as well as, antibodies directed against the
20 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:128 and may have been publicly available prior to conception
25 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1130 of SEQ ID NO:128,
30 b is an integer of 15 to 1144, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:128, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 119

The gene encoding the disclosed cDNA is thought to reside on chromosome 20. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 20.

It has been discovered that this gene is expressed primarily in Activated Neutrophils and to a lesser extent in fetal spleen, and CD34 positive cells from cord blood.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: allergies, defects in hematopoiesis and inflammation. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and hematopoiesis system the, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for reducing the allergic effects felt by allergy suffers by neutralizing the activity of the immune system, especially since neutrophils are abundant in persons suffering from allergies and other inflammatory conditions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:129 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1816 of SEQ ID NO:129, b is an integer of 15 to 1830, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:129, and where the b is greater than or
5 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 120

The translation product of this gene shares sequence homology with poly A binding protein II which is thought to be important in RNA binding for transcription
10 of RNA to DNA. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

MATPASAPDTRALVADFGYKLRQKGYVCGAGPGE
GPAADPLHQAMRAAGDEFETRFRRTFSDLAQLHVTPGSAQQRFTQVSDELF
QGGPNWGRLVAFFVFGAALCAESVNKEMEPLVGQVQEWVMVAYLETRLAD

15 WIHSSGG

WLSQITEAEMADEVICSEILSDCDSAASSPDLEELEAIKARVREMEEEAEKLKE
LQNEVEKQMNMSPPPGNAGPVMMSIEEKMEADARSIYVGNVDYGATAEELE
AHFHGCG

SVNRVTILCDKFSGHPKGFAYIEFSDKESVRTSLALDESLFRGRQIKVIPKRTN
20 RPGISTTDRGFPRARYRARTTNYNSSRSFYSGFNSRPRGRVYRGRARATSW
YSPY (SEQ ID NO: 696), VEKQMNMSPPPGNAGPVMMSIEEKM (SEQ ID NO:
697), ELEAIKARVRC (SEQ ID NO: 698),
VDYGATAEELEAHFHGCGSVNRVTILCDKFSG

HP (SEQ ID NO: 699), or ALDESLFRGRQIKVIPKRTNRPGISTTDRG (SEQ ID
25 NO: 700). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in colon and to a lesser extent in brain and immune system.

Therefore, polynucleotides and polypeptides of the invention are useful as
30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: colon cancer.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., colon, tissue and cells of the immune system, and brain or other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to poly A binding protein II indicate that polynucleotides and polypeptides corresponding to this gene are useful for detection and treatment of colon cancer and other disorders of the digestive system.. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:130 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1850 of SEQ ID NO:130, b is an integer of 15 to 1864, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:130, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 121

The translation product of this gene shares sequence homology with thymidine diphosphoglucose 4.6 dehydrase which is thought to be important in the metabolism of sugar. In specific embodiments, polypeptides of the invention comprise the

09882171.061801

PRRVEPLYIEVDQIYHLASPPNMYNPIKTLKNTIGT
LNMLGLAKRVGARLLLASTSEVYGDPEVHPQSEDYWGHVNPIGPRACYDEG
KRV

SGSQTRAFQYVSDLVNGLVALMNSNVSSPVNLGNPEEHTILEFAQLIKNLVGS
G

10 QANNQYIPKPKPARIKKGRTRHS (SEQ ID NO: 701), MLGLAKRVGAR (SEQ ID NO: 702), LLASTSEVYGDP (SEQ ID NO: 703), IGPRACYDEGKRVAET (SEQ ID NO: 704), RVARIFNT (SEQ ID NO: 705), or NDGRVVSNFI (SEQ ID NO: 706). Polynucleotides encoding these polypeptides are also encompassed by the invention. The gene encoding the disclosed cDNA is believed to reside on

This gene is expressed primarily in fetal liver and spleen and to a lesser extent in infant brain.

reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: diabetes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, spleen, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. bile, amniotic fluid, lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to thymidine diphosphoglucose 4.6 dehydrase indicate that polynucleotides and polypeptides corresponding to this gene are useful for treatment of persons with diabetes since it appears that this protein is needed in the metabolism of sugar to its more basic components. Alternatively, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 440 as residues: Ser-42 to Gly-47, Leu-62 to Pro-79, Ser-84 to Lys-89, Phe-122 to Asn-128, Pro-148 to Thr-154.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:131 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2027 of SEQ ID NO:131, b is an integer of 15 to 2041, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:131, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 122

The translation product of this gene shares sequence homology with ceruloplasmin which is thought to be important in the metabolism and transport of iron and copper. Ceruloplasmin also contains domains with homology to clotting factors V and VIII. Defects in the circulating levels of ceruloplasmin

(aceruloplasminemia) have been associated with certain disease conditions such as Wilson disease, and the accompanying hepatolenticular degeneration. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

- 5 YQAARIYYIMAEVEWDYCPDRSWEREWHNQSEKDSYGYIFLSNKDGLLGSR
RYKKAVFREYTDGTFRXPRPRTGPEEHLGILGPLIKGEVGDILTVVFKNNASR
PYSVHAHGVLESTTVWPLAAEPGEVVTYQWNIPERSGPGPMTLLVFPGSIIQ
WIPSRTCIVAWWGPWLSAKRASWXPHGGRXDMDREFALLFLIFDENKSWYL
EENVATHGSQDPGSINLQDETFLSNKMHAINGKLYANLRGLTMYQGERVA
10 WYMLAMGQDVDLHTIHFHAESFLYRNGENYRADVVDLFPGTFEVVEMVAS
NPGTWLMHCHVTDHVVHAGMETLFTVFSRTEHLSPLTVITKETEKAVPPRDIEE
GNVKMLGMQPIKQNVEMLASVLVAISVTLLLVLALGGVVWYQHRQRKLRR
NRRSILDDSFKLLSFKQ (SEQ ID NO: 707), or
GGHLSVEHPREVWPWANDSACVSWIYYSADVPIKDMYSGLVGPLA
15 ICQKGILXAPWRT (SEQ ID NO: 708). Polynucleotides encoding these
polypeptides are also encompassed by the invention.

This gene is expressed primarily in brain and retina and to a lesser extent in endothelial cells.

- Therefore, polynucleotides and polypeptides of the invention are useful as
20 reagents for differential identification of the tissue(s) or cell type(s) present in a
biological sample and for diagnosis of diseases and conditions: diseases marked by
defects in iron metabolism; aceruloplasminemia not characterized by defects in the
known ceruloplasmin gene locus; nonclassical Wilson disease; movement disorders;
and tumors derived from a brain tissue origin. Similarly, polypeptides and antibodies
25 directed to these polypeptides are useful in providing immunological probes for
differential identification of the tissue(s) or cell type(s). For a number of disorders of
the above tissues or cells, particularly of the brain, retina, and nervous system,
expression of this gene at significantly higher or lower levels may be routinely
detected in certain tissues and cell types (e.g., brain and other tissue of the nervous
30 system, retinal tissue, and endothelial cells, and cancerous and wounded tissues) or
bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another
tissue or cell sample taken from an individual having such a disorder, relative to the

standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to ceruloplasmin indicate that polynucleotides and polypeptides corresponding to this gene are useful for treatment of patients with aceruloplasminemia, or other defects in iron and/or copper metabolism. Mutations in this locus could also be diagnostic for patients currently experiencing or predicted to experience aceruloplasminemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 441 as residues: Asn-30 to Asp-37.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:132 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1998 of SEQ ID NO:132, b is an integer of 15 to 2012, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:132, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 123

The gene encoding the disclosed cDNA is believed to reside on chromosome 9. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 9.

This gene is expressed primarily in brain and B cell lymphoma and to a lesser extent in fetal liver and spleen.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune, or

developmental disorders, particularly B cell lymphoma; tumors and diseases of the brain and/or spleen; hematopoietic defects. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, blood cells, liver, and spleen, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment of disorders in neuronal, hematopoietic, and immune systems. It could potentially be useful for neurodegenerative disorders and neuronal and/or hematopoietic cell survival or proliferation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:133 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1655 of SEQ ID NO:133, b is an integer of 15 to 1669, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:133, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 124

Polynucleotides of the invention do not comprise the nucleic acid sequence shown as Genbank Accession No. gb|G20858|G20858, which is hereby incorporated herein by reference.

This gene is expressed primarily in osteoclastoma, dermatofibrosarcoma, and B cell lymphoma and to a lesser extent in endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancers, in particular osteoclastoma, dermatofibrosarcoma, and B cell lymphoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the bone, immune, and circulatory system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., bone, epidermis, blood cells, muscle, immune, and endothelial cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancers and lymphoma; osteoporosis; and the control of cell proliferation and/or differentiation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:134 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1551 of SEQ ID NO:134, b is an integer of 15 to 1565, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:134, and where the b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 125

This gene is expressed primarily in immune tissues and hematopoietic cells, particularly in activated T cells and neutrophils, spleen, and fetal liver, and to a lesser extent in infant adrenal gland.

10

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders, particularly defects in T cell activation; hematopoietic disorders, particularly tumors of hematopoietic and/or adrenal gland origin. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and/or endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., cells and tissues of the immune system, hematopoietic cells, blood cells, liver, and adrenal gland, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

15

20

25

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for immune and/or hematopoietic disorders; diseases related to proliferation and/or differentiation of hematopoietic cells; defects in T cell and neutrophil activation and responsiveness; and endocrine and/or metabolic disorders, particularly of early childhood and development. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30

09882171.061801

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 444 as residues: Met-1 to His-6.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:135 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1993 of SEQ ID NO:135, b is an integer of 15 to 2007, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:135, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 126

The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in placenta and endothelial cells and to a lesser extent in melanocytes and embryonic tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: tumors of an endothelial cell origin; angiogenesis associated with tumor development and metastasis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system and developing embryo, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, endothelial cells, melanocytes, and embryonic tissues, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having

such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment of developmental disorders; inhibition of angiogenesis; and vascular patterning. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 445 as residues: Pro-16 to Gln-21.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:136 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1277 of SEQ ID NO:136, b is an integer of 15 to 1291, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:136, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 127

This gene is expressed primarily in endothelial cells and hematopoietic tissues, including spleen, tonsils, leukocytes, and both B- and T-cell lymphomas.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders, particularly tumors of an endothelial cell and/or hematopoietic origin; leukemias and lymphomas. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and vascular systems, expression of this gene at

significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., endothelial cells, hematopoietic cells, spleen, tonsils, and blood cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the manipulation of angiogenesis; the differentiation and morphogenesis of endothelial cells; the proliferation and/or differentiation of hematopoietic cells; and the commitment of hematopoietic cells to distinct cell lineages. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:137 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1892 of SEQ ID NO:137, b is an integer of 15 to 1906, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:137, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 128

The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

It has been discovered that this gene is expressed primarily in kidney medulla and to a lesser extent in spleen from chronic myelogenous leukemia patients, prostate cancer, and some other tissues.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: tumors of a kidney origin; chronic myelogenous leukemia; prostate cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the kidney and spleen, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the diagnosis and treatment of kidney disorders and cancer, particularly chronic myelogenous leukemia and prostate cancer. It may also be useful for the enhancement of kidney tubule regeneration in the treatment of acute renal failure. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:138 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1921 of SEQ ID NO:138, b is an integer of 15 to 1935, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:138, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 129

The sequence shares homology with a rat neuroligin protein, which is thought to play a role in recognition between neurons. All neuroligins contain an N-terminal hydrophobic sequence with the characteristics of a cleaved signal peptide followed by a large esterase homology domain, a highly conserved single transmembrane region, and a short cytoplasmic domain. Neuroligins constitute a multigene family of brain-specific proteins with distinct isoforms that may have overlapping functions in mediating recognition processes between neurons. The gene encoding the disclosed cDNA is thought to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

It has been discovered that this gene is expressed primarily in adult and infant brain and to a lesser extent in mesenchymal or fibroblast cells, as well as tissues with a mesenchymal origin.

Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: tumors of a brain and/or mesenchymal origin; neurodegenerative disorders; cancer; fibrosis. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and of mesenchymal cells and tissues, expression of this gene at significantly higher or lower levels may be detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution and homology to neuroligins suggests that the protein product of this clone would be useful for the diagnosis of tumors of a brain and/or mesenchymal origin; neurodegenerative disorders; cancer; and fibrosis, based upon the expression of this gene within those tissues. Fibrosis is considered as

mesenchymal cells and fibroblasts are the primary cellular targets involved in this pathological condition.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 448 as residues: Ser-42 to Pro-47.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:139 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1432 of SEQ ID NO:139, b is an integer of 15 to 1446, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:139, and where the b is greater than or
15 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 130

This gene is expressed primarily in hepatocellular cancer and to a lesser extent in fetal tissues as well as testes tumor.

20 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: hepatic, developmental, or reproductive disorders, particularly liver cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing
25 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, fetal tissue, and testes and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids
30 (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative

to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution suggests that the protein product of this clone would be useful for the detection and treatment of liver disorders and cancers (e.g.

5 hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Similarly, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative
10 disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:140 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1095 of SEQ ID NO:140, b is an integer of 15 to 1109, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:140, and where the b is greater than or
25 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 131

This gene is expressed only in infant early brain.

Therefore, polynucleotides and polypeptides of the invention are useful as
30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: development and neural disorders, particularly neurodegenerative disorders. Similarly, polypeptides

09882171.051801

and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and nervous system, expression of this gene at significantly higher or lower levels may be routinely
 5 detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an
 10 individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating diseases of the brain in children and in treating nervous system disorders such as Alzheimer's disease, schizophrenia, dementia, depression, etc. Similarly, expression within fetal tissues and other cellular
 15 sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could
 20 again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
 25 related to SEQ ID NO:141 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
 30 general formula of a-b, where a is any integer between 1 to 483 of SEQ ID NO:141, b is an integer of 15 to 497, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:141, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 132

5 The gene encoding the disclosed cDNA is believed to reside on chromosome 14. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 14.

 This gene is expressed primarily in brain and to a lesser extent in glioblastoma.

10 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neural disorders, particularly Alzheimer's disease, schizophrenia, depression, mania, and dementia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in
15 providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids
20 (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

 The tissue distribution indicates that polynucleotides and polypeptides
25 corresponding to this gene are useful for treating brain disorders such as Alzheimer's disease, schizophrenia, depression, mania, and dementia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

 Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
30 451 as residues: Met-1 to Cys-8, Pro-10 to Gly-16, Gln-76 to Lys-89.

 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

05662171 061801
FOBT90" 1 228860

related to SEQ ID NO:142 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 255 of SEQ ID NO:142, b is an integer of 15 to 269, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:142, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 133

The translation product of this gene shares sequence homology with ribitol dehydrogenase of *Caenorhabditis elegans* which is thought to be important in metabolism of sugars, in addition to being a key enzyme in biosynthesis pathways (See Genbank Accession No. gi|1125838).

This gene is expressed primarily in macrophage and to a lesser extent in T-cell lymphoma and lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders, particularly tissue destruction in inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, blood cells and lung, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, pulmonary surfactant, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to ribitol dehydrogenase indicate that polynucleotides and polypeptides corresponding to this gene are useful for altering

macrophage metabolism in diseases such as inflammation where macrophages are known to cause excess tissue destruction. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 452 as residues: Thr-87 to Lys-93, Thr-247 to Ser-253, Pro-299 to Ser-311, Ser-315 to Arg-320.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
10 related to SEQ ID NO:143 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
15 general formula of a-b, where a is any integer between 1 to 1255 of SEQ ID NO:143, b is an integer of 15 to 1269, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:143, and where the b is greater than or equal to a + 14.

20 **FEATURES OF PROTEIN ENCODED BY GENE NO: 134**

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in pancreatic tumors, and to a lesser extent in
25 synovial sarcoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endocrine, gastrointestinal, or skeletal disorders, particularly those
30 involving proliferating tissues, such as tumors or cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of

disorders of the above tissues or cells, particularly of the endocrine and connective tissue systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., pancreas, and synovial tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating and diagnosing various cancers. Similarly, expression within pancreatic tumors and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:144 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1930 of SEQ ID NO:144, b is an integer of 15 to 1944, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:144, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 135

This gene is expressed primarily in T cell lines such as Raji and to a lesser extent in infant brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune, developmental, or neural disorders, particularly inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating and diagnosing inflammatory diseases such as rheumatoid arthritis, sepsis, inflammatory bowel disease, and psoriasis, as well as neutropenia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 454 as residues: Pro-16 to Arg-34, Gly-45 to Asn-50.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:145 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1007 of SEQ ID NO:145,

b is an integer of 15 to 1021, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:145, and where the b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 136

The translation product of this gene shares high sequence homology with SAR1 subfamily of GTP-binding proteins which is thought to be important in vesicular transport in mammalian cells. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

10 ARGGPRQPSRFAVRSPGVRPTPSRGLRSLQSQTL
SDLD (SEQ ID NO: 709), GFSSVLQFLGLYKK (SEQ ID NO: 710),
GKLVFLGLD
NAGKTTLLHMLKDDRLGQHVPTLHPT (SEQ ID NO: 711),
SEELTIAGMTFTTF
15 DLGGH (SEQ ID NO: 712), QARRVWKNYLPAINGIVFLVDCADH (SEQ ID
NO: 713), NVPILILGNKIDR (SEQ ID NO: 714), or
EVFMCSVLKRQGYGEGFRW (SEQ ID NO: 715),. Polynucleotides encoding these
polypeptides are also encompassed by the invention.

20 This gene is expressed primarily in serum-stimulated smooth muscle cells and
to a lesser extent in a T-cell lymphoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: vascular, muscular, immune, or metabolic disorders or diseases, particularly those affecting vesicular
25 transport. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the muscular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood
30 cells, and smooth muscle, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard

gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to GTP-binding proteins indicate that polynucleotides and polypeptides corresponding to this gene are useful for gene therapy in treating the large number of diseases involved in defective vesicular transport within cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 455 as residues: Lys-46 to Gln-52, Leu-108 to Leu-115, Gly-155 to Lys-160, Lys-182 to Phe-190.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:146 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1271 of SEQ ID NO:146, b is an integer of 15 to 1285, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:146, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 137

The translation product of this gene shares sequence homology with a protein found in *C. elegans* cosmid F25B5. The gene encoding the disclosed cDNA is believed to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

This gene is expressed primarily in a fetal tissues and to a lesser extent in melanocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

FOIA b 7 - D51801

biological sample and for diagnosis of diseases and conditions: abnormal fetal development, especially of the pulmonary or integumentary system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal pulmonary system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., fetal tissue, pulmonary tissue, and melanocytes, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, pulmonary surfactant, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of diseases affecting the pulmonary system, such as emphysema. Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 456 as residues: Ala-7 to Ser-15, Asp-47 to Lys-55, Tyr-160 to Val-166.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:147 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1372 of SEQ ID NO:147, b is an integer of 15 to 1386, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:147, and where the b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 138

The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

10 This gene is expressed primarily in gall bladder and to a lesser extent in smooth muscle.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: gastrointestinal,
15 digestive system, or vascular disorders, particularly atherosclerosis, vasculitis, aneurysm, and gall bladder problems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, expression of this gene at
20 significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., gall bladder and tissue of the digestive system, and smooth muscle, and cancerous and wounded tissues) or bodily fluids (e.g. bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the
25 expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating diseases of the digestive or cardiopulmonary system. Protein, as well as, antibodies directed against the protein
30 may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 457 as residues: Leu-50 to Ala-59, Pro-75 to Thr-80.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:148 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2084 of SEQ ID NO:148, b is an integer of 15 to 2098, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:148, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 139

Polypeptides of the invention do not comprise the amino acid sequence shown as Genbank accession no. W42028, which is hereby incorporated herein by reference. It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: STHASELRRRREPVGWSSRRPRGPDAAPGA GRRWCXAASGPPRAATSEPSATAGVRRRAAGPAGPGARXARRGGVELIRI (SEQ ID NO: 716). Polynucleotides encoding these polypeptides are also encompassed by the invention. The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in placenta and to a lesser extent in brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: developmental, reproductive, or neural disorders, particularly abnormal fetal development. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of developing tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating and diagnosing abnormal fetal development, reproductive, or neural disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy

targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 458 as residues: Leu-78 to Thr-88, Gly-92 to Gly-108.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:149 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1833 of SEQ ID NO:149, b is an integer of 15 to 1847, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 140

In specific embodiments, polypeptides of the invention comprise the

following amino acid sequence:

ECLDCHLHCCLLDQVVPKHYTELDASLPLRLRPES

MEKLRLCLRACVIRSLYHMYEPFAARISKNPAPIESTPSTLKNKCLLFWCRKIV
G

5 NRQEPMWEFNFKFKKQSPRLKSKCTGGLQPPVQYEDVHTNPDQDCCLLQVT
TL

NFIFIPIVMGMIFTLFTINVSTDMRHHRVRLVFQDSPVHGGRKLRSEQGVQVIL
DP

VHSVRLFDWWHPQYPFSLRA (SEQ ID NO: 717). Polynucleotides encoding these
10 polypeptides are also encompassed by the invention.

This gene is expressed primarily in smooth muscle and to a lesser extent in
ovary, prostate cancer, and activated monocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as
reagents for differential identification of the tissue(s) or cell type(s) present in a
15 biological sample and for diagnosis of diseases and conditions: vascular disorders,
particularly hypertension and atherosclerosis. Similarly, polypeptides and antibodies
directed to these polypeptides are useful in providing immunological probes for
differential identification of the tissue(s) or cell type(s). For a number of disorders of
the above tissues or cells, particularly of the circulatory system, expression of this
20 gene at significantly higher or lower levels may be routinely detected in certain
tissues and cell types (e.g., smooth muscle, ovary and other reproductive tissue,
prostate, and blood cells, and cancerous and wounded tissues) or bodily fluids
(e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or
cell sample taken from an individual having such a disorder, relative to the standard
25 gene expression level, i.e., the expression level in healthy tissue or bodily fluid from
an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides
corresponding to this gene are useful for treating diseases of the circulatory system,
such as hypertension, atherosclerosis, etc. Protein, as well as, antibodies directed
30 against the protein may show utility as a tumor marker and/or immunotherapy targets
for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:150 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1555 of SEQ ID NO:150, b is an integer of 15 to 1569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:150, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 141

Polynucleotides of the invention do not comprise the nucleic acid sequence shown as Genbank accession no. gb|G11389|G11389 which is hereby incorporated herein by reference.

This gene is expressed primarily in fetal spleen, and to a lesser extent in placenta and bone marrow.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: developmental, immune, hematopoietic, or reproductive disorders, particularly anemia and other diseases affecting blood cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the circulatory and pulmonary systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., spleen, placenta, reproductive, developmental, hematopoietic, bone marrow, and blood cells, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a

disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal spleen and bone marrow suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Alternatively, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 460 as residues: Leu-50 to Lys-58, Lys-64 to Leu-71, His-89 to Thr-94, Pro-102 to Trp-110, Tyr-162 to Cys-169, Asp-367 to Ala-377.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:151 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1526 of SEQ ID NO:151, b is an integer of 15 to 1540, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where the b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 142

The predicted translation product of this contig is a human homolog of the murine tetracycline/sugar transporter molecule recently reported by Matsuo and colleagues (Biochem. Biophys. Res. Commun. 238 (1), 126-129 (1997), incorporated herein by

10 reference). In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: LMRSIGNKNT (SEQ ID NO: 718), AWYGFGESEPC (SEQ ID NO: 719), SSITFPAVSALVSRTAD (SEQ ID NO: 720), ADQQGVVQGMITGIRGLCNGLGP
 15 ALYGFIFYIFHVELKELPITGTDLTNTSPQHHFEQN (SEQ ID NO: 721), SIIPGPPFLFGACS (SEQ ID NO: 722), FIPEHTNLSLRSSSWRKHCGRSHSPH NTQAPGEAKEP (SEQ ID NO: 723), or LLQDTNV (SEQ ID NO: 724).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in synovium and to a lesser extent in endothelial cells.

20 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: skeletal disorders, particularly rheumatoid arthritis and inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological
 25 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and lymphatic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., synovial tissue, and endothelial cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph,
 30 serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

093211061801
 1081901123350

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of inflammatory diseases, such as rheumatoid arthritis, leukemia, neutropenia, inflammatory bowel disease, psoriasis, sepsis, and the like. In addition, the protein product of this clone may also be useful for the treatment and diagnosis of a variety of autoimmune disorders, as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (ie. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 461 as residues: Leu-106 to Gln-113, Arg-153 to Lys-177.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:152 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1705 of SEQ ID NO:152, b is an integer of 15 to 1719, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:152, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 143

It is likely that the open reading frame containing the predicted signal peptide continues in the 5' direction. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: SKGNSSHSKELEASPSVVGRQPGA

VFWELWDVPLGARENRRK (SEQ ID NO: 725). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in placenta and to a lesser extent in melanocyte, fetal liver and spleen, and bone marrow.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: abnormal early development. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the
10 tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, hematopoietic, integumentary, developmental, liver, spleen, and bone marrow, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an
15 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of abnormal
20 early development phenomena and diseases. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
25 related to SEQ ID NO:153 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
30 general formula of a-b, where a is any integer between 1 to 849 of SEQ ID NO:153, b is an integer of 15 to 863, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:153, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 144

Polynucleotides of the invention do not comprise the nucleic acid sequence shown as Genbank accession no. gb|G15249|G15249, which is hereby incorporated herein by reference.

This gene is expressed primarily in fetal liver and spleen.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: developmental or immune disorders, particularly anemia and neutropenia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and blood systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver and spleen, developmental, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g. amniotic fluid, lymph, bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful in hematopoiesis and bone marrow regeneration as it is most abundant in fetal tissues responsible for the generation of hematopoietic cells. Similarly, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein

09882171-051801

may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 463 as residues: Ser-35 to Lys-46.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:154 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1087 of SEQ ID NO:154, b is an integer of 15 to 1101, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:154, and where the b is greater than or
15 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 145

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in
20 linkage analysis for chromosome 3.

The translation product of this gene shares sequence homology with protein tyrosine phosphatase which is thought to be important in transducing signals to activate cells such as T cell, B cell and other cell types.

This gene is expressed primarily in T cells and tissues in early stages of
25 development and to a lesser extent in cancers.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immuno-related diseases and cancer. Similarly, polypeptides and antibodies directed to these
30 polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system expression of this gene at

significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., embryonic and fetal tissue, undifferentiated cells, and blood cells, immune tissues, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to the protein tyrosine phosphatase suggests that the protein product of this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Expression of this gene product in T-cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

09082171.061901

related to SEQ ID NO:155 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2017 of SEQ ID NO:155, b is an integer of 15 to 2031, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:155, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 146

This gene is expressed primarily in T cell and to a lesser extent in B cell, macrophages and tumor tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immuno-disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, blood cells, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for regulating the immune system and therefore can be used in treating diseases such as autoimmune diseases and cancers. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:156 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1967 of SEQ ID NO:156, b is an integer of 15 to 1981, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:156, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 147

This gene is expressed primarily in placenta, and to a lesser extent in endothelial cells, testis tumor, ovarian cancer, uterine cancer.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, endothelial cells, testis and ovary and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancers or reproductive disorders. Protein, as well as, antibodies directed against the protein may

show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 466 as residues: Lys-136 to Thr-145.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:157 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 901 of SEQ ID NO:157, b is an integer of 15 to 915, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:157, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 148

This sequence has significant homology to mouse torsin A. Recently, another group cloned the human Torsin A gene. (See, Accession No. 2358279; see also Nature Genet. 17, 40-48 (1997), incorporated herein by reference.) In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: VEPISLGLALAGVLTGYIYP (SEQ ID NO: 726), PLTSLHGWGTGTGKNFVSKIIAENIYE (SEQ ID NO: 727), NITLYKDQLQLW IRGNVSACARSI (SEQ ID NO: 728), DFWRSGKQREDIKLKDIEHALSVSVFN (SEQ ID NO: 729), LIDYFVPFLPLEYKHLKMCIRV (SEQ ID NO: 730), VSRVAEEMTF FPKEERVF (SEQ ID NO: 731). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in osteoclastoma, T-cell, and placenta and to a lesser extent in fetal lung, fetal liver, fetal brain, adult brain and tumor tissues

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: disease conditions in

T08T90 "T2T8860

hematopoiesis and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoiesis system, expression of this gene at

5 significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, bone, placenta, lung, liver, and brain and other tissues of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression

10 level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating blood related diseases such as deficiencies in red blood cell, white blood cell, platelet and other hematopoiesis cells.

15 Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 467 as residues: Cys-50 to Ser-57, Asn-88 to Pro-95, Trp-222 to Lys-232, Asn-246 to Phe-252, Gln-286 to Ile-292, Asp-327 to Asp-332.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:158 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2103 of SEQ ID NO:158, b is an integer of 15 to 2117, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:158, and where the b is greater than or

30 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 149

The gene encoding the disclosed cDNA is believed to reside on chromosome 6. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 6.

This gene is expressed primarily in T cell, prostate and prostate cancer, endothelial cells and to a lesser extent in monocyte, dendritic cell, bone marrow, salivary gland, colon cancer, stomach cancer, pancreatic tumor, uterine cancer, fetal spleen and osteoclastoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immuno-related diseases and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., blood cells, prostate, endothelial cells, dendritic cells, bone marrow, salivary gland, colon, stomach, pancreas, uterus, spleen and bone, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment of cancers. Similarly, the tissue distribution within T-cells, dendritic cells, and bone marrow suggests that the protein product of this clone would be useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in

the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:159 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2381 of SEQ ID NO:159, b is an integer of 15 to 2395, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:159, and where the b is greater than or
15 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 150

This gene was recently cloned by another group, calling it eIF3-p66. (See Accession No. 2351378.) This gene plays a role in RNA binding and macromolecular
20 assembly, and therefore, any mutations in this gene would likely result in a diseased phenotype. Preferred polypeptide fragments comprise the amino acid sequence:

MAKFMTPIQDNPSGWGPCAVPEQFRDMPYQPFSKGDRLGKVADWTGATY
QDKRYTNKYSSQFGGGSQYAYFHEEDESSFQLVDTARTQKTAYQRNRMRF
QRNLRRDKDRNMLQFNLQILPKSAKQKERERIRLQKKFQKQFGVRQKWDQ
25 KSQKPRDSSVEVRSDWEVKEEMDFPQLMKMRYLEVSEPQDIECCGALEYD
KAFDRITRSEKPLRXXKRIFHTVTTTDDPVIRKLAKTQGNVFATDAILATLM
SCTRSVYSWDIVVQRVGSKLFFDKRDNSDFDLLTVSETANEPPQDEGNSFNSP
RNLAMEATYINHNFSQQCLRMGKERYNFPNPNPFVEDDMDKNEIASVAYRY
RSGKLGDDIDLIVRCEHDGVMTGANGEVSFINIKTLNEWDSRHCNGVDWRQ
30 KLDSQRGAVIATELKNNSYKLARWTCCALLAGSEYKLKGYVSRVYHVKDSSR
HVILGTQQFKPNEFASQINLSVENAWGILRCVIDICMKLEEGKYLILKDPNKQ
VIRVYSLPDGTFSS (SEQ ID NO: 732), or

CQSHLSRLGASEANEDAWRNAVTDFRVDLRFTAREFFRPTQRKRESSLRQRR
SC

LRRRPLRAHVSSFPGFSRVLLLTRSPARTICCHPGRPRHCRFWK (SEQ ID NO:
733), as well as N-terminal and C-terminal deletions of this polypeptide fragment.

5 This gene is expressed primarily in T cell, bone marrow, embryo and
endothelial cells and to a lesser extent in testis tumor and endometrial tumor.

Therefore, polynucleotides and polypeptides of the invention are useful as
reagents for differential identification of the tissue(s) or cell type(s) present in a
biological sample and for diagnosis of diseases and conditions: immune disorders,
10 particularly inflammatory or immunodeficiency disorders, and tumors. Similarly,
polypeptides and antibodies directed to these polypeptides are useful in providing
immunological probes for differential identification of the tissue(s) or cell type(s). For
a number of disorders of the above tissues or cells, particularly of the immune system
and reproductive system, expression of this gene at significantly higher or lower
15 levels may be routinely detected in certain tissues and cell types (e.g. immune,
developmental, hematopoietic, and cancerous and wounded tissues) or bodily fluids
(e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or
another tissue or cell sample taken from an individual having such a disorder, relative
to the standard gene expression level, i.e., the expression level in healthy tissue or
20 bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides
corresponding to this gene are useful for immune disorders and cancers. Similarly,
expression within embryonic tissue and other cellular sources marked by proliferating
cells suggests that this protein may play a role in the regulation of cellular division,
25 and may show utility in the diagnosis and treatment of cancer and other proliferative
disorders. Similarly, embryonic development also involves decisions involving cell
differentiation and/or apoptosis in pattern formation. Thus this protein may also be
involved in apoptosis or tissue differentiation and could again be useful in cancer
therapy. Protein, as well as, antibodies directed against the protein may show utility
30 as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
469 as residues: Gln-10 to Trp-16, Pro-32 to Leu-39, Tyr-50 to Gln-68, Ala-86 to

05882171.051801

Phe-100, Arg-103 to Asn-113, Ser-125 to Arg-133, Gln-137 to Lys-142, Arg-147 to Ser-161, Arg-165 to Glu-173, Ile-209 to Arg-218.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:160 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2106 of SEQ ID NO:160, b is an integer of 15 to 2120, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:160, and where the b is greater than or equal to a + 14.

09882171.061801
FOOTNOTES

FEATURES OF PROTEIN ENCODED BY GENE NO: 151

The translation product of this gene was found to have homology to the human

VRK1 and VRK2 proteins (See Genbank Accession No. gi|1827450 (AB000449),

5 and gi|1827452 (AB000450), respectively) which are thought to encode novel serine/threonine kinases. Based upon the role of such proteins in regulation of various cellular processes, namely regulation of cellular proliferation, an important function for this clone would be realized by a skilled artisan. In specific embodiments, polypeptides of the invention comprise the following amino acid
10 sequence:

KLKSFQTRDNQGILYEAAPTSTLTCXSGPQKQKFSCLKLDAKDGRLEFNEQNFFQ
RAAKPLQVKNWKKLYSTPLLAIPTCMGFGVHQDKYRFLVLPSTLGRSL
QSALDVSPKHVLCREVC AAGGLPAAGCPGVPP (SEQ ID NO: 734).

Polynucleotides

15 encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testes and to a lesser extent in T cell, spinal cord, placenta, neutrophil and monocyte.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a
20 biological sample and for diagnosis of diseases and conditions: male reproductive and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive, immune and endocrine systems,
25 expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testis and other reproductive tissue, blood cells, tissue of the nervous system, and placenta, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, seminal fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having
30 such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for regulating immune and reproductive functions.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 470 as residues: Lys-2 to Trp-24, Arg-35 to Gly-40, Lys-46 to Cys-58, Arg-71 to Trp-77, Glu-164 to Gly-172.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:161 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 886 of SEQ ID NO:161, b is an integer of 15 to 900, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:161, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 152

The translation product of this gene shares sequence homology with tyrosyl-tRNA synthetase which is thought to be important in cell growth.

This gene is expressed primarily in brain, liver, keratinocytes, tonsils, and heart.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and/or autoimmune diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, liver, keratinocytes,

tonsils and heart, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissues of the nervous system, liver, keratinocytes, tonsils and heart, immune, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to tyrosyl-tRNA synthetase indicate that polynucleotides and polypeptides corresponding to this gene are useful for modulating cell growth. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 471 as residues: Thr-2 to Glu-17, Cys-74 to Ser-79.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:162 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 989 of SEQ ID NO:162, b is an integer of 15 to 1003, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:162, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 153

This gene is homologous to the Drosophila transcriptional regulator dre4. (See Genbank Accession No. 2511745.) Dre4 is a gene required for steroidogenesis in Drosophila melanogaster and encodes a developmentally expressed homologue of the yeast transcriptional regulator CDC68. Preferred polypeptide fragments comprise the amino acid sequence:

KKRHTDVQFYTEVGEITTDLGKHQHMHDRDDLYAEQMEREMRHKLKTAFK
 NFIEKVEALTKEELEFEVPRDLGFNGAPYRSTCLLQPTSSALVNATEWPPFV
 VTLDEVELIHFVRVQFHLKNFDMVIVYKDYSSKKVTMINAIPVASLDPIKEWLN
 SCDLKYTEGVQSLNWTIMKTIVDDPEGFFEQQGGWSFL (SEQ ID NO: 735), as

5 well as N-terminal and C-terminal deletions of this fragments. Also preferred are polynucleotide fragments encoding this polypeptide fragment.

This gene is expressed primarily in fetal liver, spleen, placenta, lung, T cell, thyroid, testes.

Therefore, polynucleotides and polypeptides of the invention are useful as
 10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: brain tumor, heart and liver diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,
 15 particularly of the fetal liver, spleen, placenta, lung, T cell, thyroid and testes, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, spleen, placenta, lung, blood cells, thyroid, and testes and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or
 20 another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal liver, spleen, T-cells, and thyroid suggests that the protein product of this clone would be useful for the diagnosis and treatment of a
 25 variety of immune system disorders. Expression of this gene product in immune cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of
 30 cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including

05832171.061804

arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:163 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2182 of SEQ ID NO:163, b is an integer of 15 to 2196, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:163, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 154

The translation product of this gene was found to have homology to both the human cAMP-dependent protein kinase and phospholemman chloride channel, which are thought to be involved in regulation of signal transduction pathways, potentially for cell cycle regulation (See Genbank Accession No. pir|A40533|A40533 and gi|1916010, respectively). The gene encoding the disclosed cDNA is believed to reside on

chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed primarily in brain and to a lesser extent in fetal heart, testis, spleen, lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: heart, liver and spleen diseases, immunological diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, fetal heart, testis, spleen and lung, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, heart, testes and other reproductive tissue, spleen, and lung, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain combined with the homology to the conserved cAMP-dependent protein kinase and phospholemman proteins suggests that the protein product of this clone would be useful for the detection/treatment of neurodegenerative

disease states, behavioural disorders, or inflammatory conditions such as Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system.

09882171.061801
FOBT90"TZ28860

Alternatively, expression within fetal tissues and other cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 473 as residues: Glu-22 to Phe-30, Arg-59 to Ala-77, Ala-87 to Asn-95.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:164 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1931 of SEQ ID NO:164, b is an integer of 15 to 1945, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:164, and where the b is greater than or equal to a + 14.

25 FEATURES OF PROTEIN ENCODED BY GENE NO: 155

Activation of T cells through the T cell antigen receptor (TCR) results in the rapid tyrosine phosphorylation of a number of cellular proteins, one of the earliest being a 100 kDa protein. This gene is the human equivalent of murine valosin containing protein (VCP). VCP is a member of a family of ATP binding, homo-oligomeric proteins, and the mammalian homolog of *Saccharomyces cerevisiae* cdc48p, a protein essential to the completion of mitosis in yeast. Both endogenous and expressed murine VCP are tyrosine phosphorylated in response to T cell

activation. Thus we have identified a novel component of the TCR mediated tyrosine kinase activation pathway that may provide a link between TCR activation and cell cycle control. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence: MASGADSKGDDLSTAILKQKN

- 5 RPNRLIVDEAINEDN (SEQ ID NO:736), GKKRREAVCIVLSDDTCSDEKIRM
(SEQ ID NO: 737), GDVISIQPCPDVKYGKRIHVLPIID (SEQ ID NO: 738),
ITGNLFEVYL
KPYFLEAYRPIRKGDIFLVRGGMRA (SEQ ID NO: 739),
GYDDIGGCRKQLAQIKE
10 MVELPLRHPALFKAI (SEQ ID NO: 740),
PPGTGKTLIARAVANETGAFFFLINGPEI
MSKLAGE (SEQ ID NO: 741),
RRIVSQLLTLM DGLKQRAHVIVMAATNRPNSIDP
A (SEQ ID NO: 742), VDIPDATGRLEILQIHTKNMKLADDVD (SEQ ID NO:
15 743), EAALQAIRKKMDLIDLEDETI (SEQ ID NO: 744),
TVVEVPQVTWEDIGGLEDVKREL
QELVQYPVEHPD (SEQ ID NO: 745),
NECQANFISIKGPELLTMWFGGESEANVREIF
DKAR (SEQ ID NO: 746), ILTEMDGMSTKKNVFIIGATNRPDIIDPAI (SEQ ID
20 NO: 747), IYIPLPDEKSRVAILKANLRKSPVAKDVDLEFL (SEQ ID NO: 748),
DPVPEIRR
DHFEAMRFAARRSVSDNDI (SEQ ID NO: 749), QTLQQSRGFGSFRFP (SEQ ID
NO: 750), or
CGPGTVANETHGHVGADLAALCSEAALQAIRKKMDLIDLEDETIDAEVMN
25 SLAVTMDDFRWALSQSNPSALRETVEVPQVTWEDIGGLEDVKRELQELVQ
YPVEHPDKFLKFGMTPSKGVLFYGPFGCGKTLLAKAIA NECQANFISIKGPEL
LTMWFG
ESEANVREIFDKARQAAPCVLFFDELDSLAKARGGNIGDGGGAADRVINQILT
EMDGMSTKKNVFIIGATNRPDIIDPAILRPGRLDQLIYIPLPDEKSRVAILKANL
30 RKSPVA

KDVDLEFLAKMTNGFSGADLTEICQRACKLAIESIESEIRRERERQTNPSAME
VEEDDPVPEIRRDHFEEAMRFARRSVSDNDIRKYEMFAQTLQQSRGFGSFRFP
SGNQG

GAGPSQGSGGGTGGSVYTEDNDDDLYG (SEQ ID NO: 751). Polynucleotides

5 encoding these polypeptides are also encompassed by the invention. The gene encoding the disclosed cDNA is believed to reside on chromosome 9. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 9.

This gene is expressed primarily in brain, liver, spleen, placenta.

10 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological
15 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, liver, spleen, placenta expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, liver, spleen, and placenta, and cancerous and wounded tissues) or bodily
20 fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to VCR indicate that polynucleotides
25 and polypeptides corresponding to this gene are useful for treating cancer. The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are cytokine, cell
30 proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy);

05882171.061801

regulation of hematopoiesis (e.g. for treating anaemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors);

5 hemostatic or thrombolytic activity (e.g. for treating haemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behaviour. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies

10 directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 474 as residues: Ala-5 to Asp-11, Lys-18 to Asn-24, Asp-75 to Ile-82, Pro-106 to Lys-112, Arg-144 to Gly-149, Glu-187 to Ser-197, Gly-202 to Cys-209, Ser-282 to

15 Lys-288, Glu-291 to Asn-296, Pro-311 to His-317, Thr-347 to Ser-352, Arg-359 to Val-367.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:165 and may have been publicly available prior to conception

20 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2919 of SEQ ID NO:165,

25 b is an integer of 15 to 2933, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:165, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 156

30 The translation product of this gene shares sequence homology with rat growth response protein which is thought to be important in cell growth. A group recently cloned the human homolog of this gene, calling it insulin induced protein 1.

(See Accession No. 2358269, see also, Genomics 43 (3), 278-284 (1997).) Preferred polypeptide fragments comprise the amino acid sequence:

RSGLGLGITIAFLATLITQFLVYNGVYQYTSPDF

LYIRSWLPCIFFSGGVTVGNIGRQLAMGVPEKPHSD (SEQ ID NO: 752), as well

5 as N-terminal and C-terminal deletions of this polypeptide fragment. Also preferred are polynucleotide fragments encoding these polypeptide fragments. The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

10 This gene is expressed primarily in brain, liver, placenta, heart, spleen, lymphoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are
 15 not limited to, cancer and immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, liver, placenta, heart, spleen, expression of this gene at significantly higher or lower levels may be routinely
 20 detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, liver, placenta, heart, spleen, and lymphoid tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in
 25 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in liver and homology to growth-response protein indicate that polynucleotides and polypeptides corresponding to this gene are useful for modulating cell growth and may play a role in growth and differentiation of tissues involved in metabolic control or development. Protein, as well as, antibodies
 30 directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

D58821.1 061801

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:166 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2229 of SEQ ID NO:166, b is an integer of 15 to 2243, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:166, and where the b is greater than or equal to a + 14.

05682171.061801

FEATURES OF PROTEIN ENCODED BY GENE NO: 157

The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

5 This gene is expressed primarily in Glioblastoma, endometrial tumor, lymphoma and pancreas tumor.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: Glioblastoma,
10 Endometrial tumor, lymphoma and pancreas tumor, and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, expression of this gene at significantly higher or lower levels may be
15 routinely detected in certain tissues and cell types (e.g., endometrium, lymphoid tissue, pancreas, and tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, bile, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in
20 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in tumors of pancreas, endometrium, and other proliferative tissues suggests that the protein product of this clone would be useful for diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the
25 protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 476 as residues: Thr-60 to Glu-66, Tyr-104 to Tyr-111.

Many polynucleotide sequences, such as EST sequences, are publicly
30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:167 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1802 of SEQ ID NO:167, b is an integer of 15 to 1816, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:167, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 158

The translation product of this gene shares sequence homology with IGE receptor which is thought to be important in allergy and asthma. In specific embodiments, polypeptides of the invention comprise the following amino acid sequence:

ETRVKTSLELLRTQLEPTGTVGNTINFSQAEKPEPTNQGQDSLKKHLHAEIKVI

GTIQILCG (SEQ ID NO: 753). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in T cell, and fetal liver.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: allergy and asthma and other immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g. immune, blood cells, and liver, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to IgE receptor indicate that polynucleotides and polypeptides corresponding to this gene are useful for allergy and

asthma. Similarly, The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are

5 cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anaemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for
10 treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating haemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for
15 regulation of metabolism, and behaviour. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
20 477 as residues: Glu-94 to Thr-102.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:168 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
25 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 931 of SEQ ID NO:168, b is an integer of 15 to 945, where both a and b correspond to the positions of
30 nucleotide residues shown in SEQ ID NO:168, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 159

For purposes of this application, this gene and its corresponding translation product are known as the B7-H5 gene and B7-H5 protein. This protein is believed to reside as a cell-surface molecule, and the transmembrane domain of this protein is believed to embody the following preferred amino acid residues:

IRVPVFNIVILLAGGF (SEQ ID NO: 758). Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these peptides. The B7-H5 gene shares sequence homology with members of the B7 family of ligands (i.e., B7-1 (See Genbank Accession 507873)). These proteins and their corresponding receptors play vital roles in the growth, differentiation and death of T cells. For example, some members of this family (i.e., B7-H1) are involved in costimulation of the T cell response, as well as inducing increased cytokine production. Therefore, antagonists such as antibodies or small molecules directed against the B7-H5 gene are useful for treating T cell mediated immune system disorders. The gene encoding the disclosed cDNA is thought to reside on chromosome 6. Accordingly, polynucleotides related to this invention have uses, such as, for example, as a marker in linkage analysis for chromosome 6.

It has been discovered that this gene is expressed in activated neutrophils and activated T cells, and to a lesser extent in monocytes and heart tissue.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven or all seven of the immunogenic epitopes of the extracellular portion of the B7-H5 protein shown in SEQ ID NO: 478 as residues: Leu-24 to Gln-35, Arg-59 to Pro-64, Glu-71 to His-78, Asp-89 to Gly-94, Pro-141 to Val-151, Thr-167 to Val-172, Ala-175 to Thr-180. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these peptides.

In additional nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, one or more of the following amino acid sequences:

1.) The extracellular domain of the B7-H5 protein:

MRKTRLWGLLWMLFVSELRAATKLTEEKYELKEGQTLDVKCDYTLKFASS
QKA WQIIRDGEMP KTLACTERPSKNSHPVQVGRILEDYHDHGLLRVRMVNL

09882171.061801

QVEDSGLYQCVIYQPPKEPHMLFDRIRLVVTKGFSGTPGSNENSTQNVYKIPP
TTTKALCPLYTSPRTVTQAPPKSTADVSTPDSEINLTNVTDI (SEQ ID NO:
759),

2.) The mature extracellular domain of the B7-H5 protein:

5 EKYELKEGQTLDDVKCDYTLEKFASSQKAWQIIRDGEMPCTLACTERPSKNSH
PVQVGRILEDYHHDHGLLRVRMVNLQVEDSGLYQCVIYQPPKEPHMLFDRIR
LVVTKGFSGTPGSNENSTQNVYKIPPTTTKALCPLYTSPRTVTQAPPKSTADV
STPDSEINLTNVTDI (SEQ ID NO: 760), and/or

3.) The leader sequence of the B7-H5 protein:

10 MRKTRLWGLLWMLFVSELRAATKLTE (SEQ ID NO: 761).

Polynucleotides encoding these polypeptides are also encompassed by the invention,
as are antibodies that bind one or more of these polypeptides.

Also preferred are polypeptides comprising, or alternatively consisting of,
fragments of the mature extracellular portion of the B7-H5 protein demonstrating
15 functional activity (SEQ ID NO: 760). Polynucleotides encoding these polypeptides
are also encompassed by the invention. By functional activity is meant, a polypeptide
fragment capable of displaying one or more known functional activities associated
with the full-length (complete) B7-H5 protein. Such functional activities include, but
are not limited to, biological activity (e.g., T cell costimulatory activity, ability to bind
20 ICOS, and ability to induce or inhibit cytokine production), antigenicity [ability to
bind (or compete with a B7-H5 polypeptide for binding) to an anti-B7-H5 antibody],
immunogenicity (ability to generate antibody which binds to a B7-H5 polypeptide),
ability to form multimers with B7-H5 polypeptides of the invention, and ability to
bind to a receptor or ligand for a B7-H5 polypeptide.

25 Figure 1 shows the nucleotide (SEQ ID NO: 169) and deduced amino acid
sequence (SEQ ID NO: 478) corresponding to this gene.

Figure 2 shows an analysis of the amino acid sequence (SEQ ID NO: 478).
Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic
regions; flexible regions; antigenic index and surface probability are shown, and all
30 were generated using the default settings of the recited computer algorithms. In the
"Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the
highly antigenic regions of the protein, i.e., regions from which epitope-bearing

peptides of the invention can be obtained. Polypeptides comprising, or alternatively consisting of, domains defined by these graphs are contemplated by the present invention, as are polynucleotides encoding these polypeptides.

The data presented in Figure 2 are also represented in tabular form in Table 3.

- 5 The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 2, and Table 3: "Res": amino acid residue of SEQ ID NO: 478 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO: 478 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

- Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consisting of, one or more of the following regions: alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions. The data representing the structural or functional attributes of the protein set forth in Figure 2 and/or Table 3, as described above, was generated using the various modules and algorithms of the DNA*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table 3 can be used to determine regions of the protein which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figure 2, but may, as shown in Table 3, be represented or identified by using tabular representations of the data presented in Figure 2. The DNA*STAR computer algorithm used to generate Figure 2 (set on the original default parameters) was used to present the data in Figure 2 in a tabular format (See Table 3). The tabular format of the data in Figure 2 is used to easily determine specific boundaries of a preferred region.

The present invention is further directed to fragments of the polynucleotide sequences described herein. By a fragment of, for example, the polynucleotide sequence of a deposited cDNA or the nucleotide sequence shown in SEQ ID NO: 169, is intended polynucleotide fragments at least about 15nt, and more preferably at least about 20 nt, at least about 25nt, still more preferably at least about 30 nt, at least about 35nt, and even more preferably, at least about 40 nt in length, at least about 45nt in length, at least about 50nt in length, at least about 60nt in length, at least about 70nt in length, at least about 80nt in length, at least about 90nt in length, at least about 100nt in length, at least about 125nt in length, at least about 150nt in length, at least about 175nt in length, which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 200-1500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the nucleotide sequence of a deposited cDNA or as shown in SEQ ID NO: 169. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO: 169. In this context "about" includes the particularly recited size, an sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Representative examples of polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to about 50, from about 51 to about 100, from about 101 to about 150, from about 151 to about 200, from about 201 to about 250, from about 251 to about 300, from about 301 to about 350, from about 351 to about 400, from about 401 to about 450, from about 451 to about 500, and from about 501 to about 550, and from about 551 to about 600, from about 601 to about 650, from about 651 to about 700, from about 701 to about 750, from about 751 to about 800, and from about 801 to about 860, of SEQ ID NO:

169, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In additional embodiments, the polynucleotides of the invention encode
 5 functional attributes of the corresponding protein.

Preferred polypeptide fragments of the invention comprise, or alternatively consist of, the secreted protein having a continuous series of deleted residues from the amino or the carboxy terminus, or both. Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-234 where m is an integer
 10 from 2 to 228, where m corresponds to the position of the amino acid residue identified in SEQ ID NO: 478. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: R-2 to P-234; K-3 to P-234; T-4 to P-234; R-5 to P-234; L-6 to P-234; W-7 to P-234; G-8 to P-234; L-9 to P-234; L-10 to
 15 P-234; W-11 to P-234; M-12 to P-234; L-13 to P-234; F-14 to P-234; V-15 to P-234; S-16 to P-234; E-17 to P-234; L-18 to P-234; R-19 to P-234; A-20 to P-234; A-21 to P-234; T-22 to P-234; K-23 to P-234; L-24 to P-234; T-25 to P-234; E-26 to P-234; E-27 to P-234; K-28 to P-234; Y-29 to P-234; E-30 to P-234; L-31 to P-234; K-32 to P-234; E-33 to P-234; G-34 to P-234; Q-35 to P-234; T-36 to P-234; L-37 to P-234;
 20 D-38 to P-234; V-39 to P-234; K-40 to P-234; C-41 to P-234; D-42 to P-234; Y-43 to P-234; T-44 to P-234; L-45 to P-234; E-46 to P-234; K-47 to P-234; F-48 to P-234; A-49 to P-234; S-50 to P-234; S-51 to P-234; Q-52 to P-234; K-53 to P-234; A-54 to P-234; W-55 to P-234; Q-56 to P-234; I-57 to P-234; I-58 to P-234; R-59 to P-234; D-60 to P-234; G-61 to P-234; E-62 to P-234; M-63 to P-234; P-64 to P-234; K-65 to
 25 P-234; T-66 to P-234; L-67 to P-234; A-68 to P-234; C-69 to P-234; T-70 to P-234; E-71 to P-234; R-72 to P-234; P-73 to P-234; S-74 to P-234; K-75 to P-234; N-76 to P-234; S-77 to P-234; H-78 to P-234; P-79 to P-234; V-80 to P-234; Q-81 to P-234; V-82 to P-234; G-83 to P-234; R-84 to P-234; I-85 to P-234; I-86 to P-234; L-87 to P-234; E-88 to P-234; D-89 to P-234; Y-90 to P-234; H-91 to P-234; D-92 to P-234; H-
 30 93 to P-234; G-94 to P-234; L-95 to P-234; L-96 to P-234; R-97 to P-234; V-98 to P-234; R-99 to P-234; M-100 to P-234; V-101 to P-234; N-102 to P-234; L-103 to P-234; Q-104 to P-234; V-105 to P-234; E-106 to P-234; D-107 to P-234; S-108 to P-

234; G-109 to P-234; L-110 to P-234; Y-111 to P-234; Q-112 to P-234; C-113 to P-234; V-114 to P-234; I-115 to P-234; Y-116 to P-234; Q-117 to P-234; P-118 to P-234; P-119 to P-234; K-120 to P-234; E-121 to P-234; P-122 to P-234; H-123 to P-234; M-124 to P-234; L-125 to P-234; F-126 to P-234; D-127 to P-234; R-128 to P-234; I-129 to P-234; R-130 to P-234; L-131 to P-234; V-132 to P-234; V-133 to P-234; T-134 to P-234; K-135 to P-234; G-136 to P-234; F-137 to P-234; S-138 to P-234; G-139 to P-234; T-140 to P-234; P-141 to P-234; G-142 to P-234; S-143 to P-234; N-144 to P-234; E-145 to P-234; N-146 to P-234; S-147 to P-234; T-148 to P-234; Q-149 to P-234; N-150 to P-234; V-151 to P-234; Y-152 to P-234; K-153 to P-234; I-154 to P-234; P-155 to P-234; P-156 to P-234; T-157 to P-234; T-158 to P-234; T-159 to P-234; K-160 to P-234; A-161 to P-234; L-162 to P-234; C-163 to P-234; P-164 to P-234; L-165 to P-234; Y-166 to P-234; T-167 to P-234; S-168 to P-234; P-169 to P-234; R-170 to P-234; T-171 to P-234; V-172 to P-234; T-173 to P-234; Q-174 to P-234; A-175 to P-234; P-176 to P-234; P-177 to P-234; K-178 to P-234; S-179 to P-234; T-180 to P-234; A-181 to P-234; D-182 to P-234; V-183 to P-234; S-184 to P-234; T-185 to P-234; P-186 to P-234; D-187 to P-234; S-188 to P-234; E-189 to P-234; I-190 to P-234; N-191 to P-234; L-192 to P-234; T-193 to P-234; N-194 to P-234; V-195 to P-234; T-196 to P-234; D-197 to P-234; I-198 to P-234; I-199 to P-234; R-200 to P-234; V-201 to P-234; P-202 to P-234; V-203 to P-234; F-204 to P-234; N-205 to P-234; I-206 to P-234; V-207 to P-234; I-208 to P-234; L-209 to P-234; L-210 to P-234; A-211 to P-234; G-212 to P-234; G-213 to P-234; F-214 to P-234; L-215 to P-234; S-216 to P-234; K-217 to P-234; S-218 to P-234; L-219 to P-234; V-220 to P-234; F-221 to P-234; S-222 to P-234; V-223 to P-234; L-224 to P-234; F-225 to P-234; A-226 to P-234; V-227 to P-234; T-228 to P-234; and/or L-229 to P-234 of SEQ ID NO:478. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the following group of C-terminal deletions: M-1 to V-233; M-1 to F-232; M-1 to S-231; M-1 to R-230; M-1 to L-229; M-1 to T-228; M-1 to V-227; M-1 to A-226; M-1 to F-225; M-1 to L-224; M-1 to V-223; M-1 to S-222; M-1 to F-221; M-1 to V-220; M-1 to L-219; M-1 to S-218; M-1 to K-217; M-1 to S-216; M-1 to L-215; M-1 to F-214;

M-1 to G-213; M-1 to G-212; M-1 to A-211; M-1 to L-210; M-1 to L-209; M-1 to I-208; M-1 to V-207; M-1 to I-206; M-1 to N-205; M-1 to F-204; M-1 to V-203; M-1 to P-202; M-1 to V-201; M-1 to R-200; M-1 to I-199; M-1 to I-198; M-1 to D-197; M-1 to T-196; M-1 to V-195; M-1 to N-194; M-1 to T-193; M-1 to L-192; M-1 to N-191; M-1 to I-190; M-1 to E-189; M-1 to S-188; M-1 to D-187; M-1 to P-186; M-1 to T-185; M-1 to S-184; M-1 to V-183; M-1 to D-182; M-1 to A-181; M-1 to T-180; M-1 to S-179; M-1 to K-178; M-1 to P-177; M-1 to P-176; M-1 to A-175; M-1 to Q-174; M-1 to T-173; M-1 to V-172; M-1 to T-171; M-1 to R-170; M-1 to P-169; M-1 to S-168; M-1 to T-167; M-1 to Y-166; M-1 to L-165; M-1 to P-164; M-1 to C-163; M-1 to L-162; M-1 to A-161; M-1 to K-160; M-1 to T-159; M-1 to T-158; M-1 to T-157; M-1 to P-156; M-1 to P-155; M-1 to I-154; M-1 to K-153; M-1 to Y-152; M-1 to V-151; M-1 to N-150; M-1 to Q-149; M-1 to T-148; M-1 to S-147; M-1 to N-146; M-1 to E-145; M-1 to N-144; M-1 to S-143; M-1 to G-142; M-1 to P-141; M-1 to T-140; M-1 to G-139; M-1 to S-138; M-1 to F-137; M-1 to G-136; M-1 to K-135; M-1 to T-134; M-1 to V-133; M-1 to V-132; M-1 to L-131; M-1 to R-130; M-1 to I-129; M-1 to R-128; M-1 to D-127; M-1 to F-126; M-1 to L-125; M-1 to M-124; M-1 to H-123; M-1 to P-122; M-1 to E-121; M-1 to K-120; M-1 to P-119; M-1 to P-118; M-1 to Q-117; M-1 to Y-116; M-1 to I-115; M-1 to V-114; M-1 to C-113; M-1 to Q-112; M-1 to Y-111; M-1 to L-110; M-1 to G-109; M-1 to S-108; M-1 to D-107; M-1 to E-106; M-1 to V-105; M-1 to Q-104; M-1 to L-103; M-1 to N-102; M-1 to V-101; M-1 to M-100; M-1 to R-99; M-1 to V-98; M-1 to R-97; M-1 to L-96; M-1 to L-95; M-1 to G-94; M-1 to H-93; M-1 to D-92; M-1 to H-91; M-1 to Y-90; M-1 to D-89; M-1 to E-88; M-1 to L-87; M-1 to I-86; M-1 to I-85; M-1 to R-84; M-1 to G-83; M-1 to V-82; M-1 to Q-81; M-1 to V-80; M-1 to P-79; M-1 to H-78; M-1 to S-77; M-1 to N-76; M-1 to K-75; M-1 to S-74; M-1 to P-73; M-1 to R-72; M-1 to E-71; M-1 to T-70; M-1 to C-69; M-1 to A-68; M-1 to L-67; M-1 to T-66; M-1 to K-65; M-1 to P-64; M-1 to M-63; M-1 to E-62; M-1 to G-61; M-1 to D-60; M-1 to R-59; M-1 to I-58; M-1 to I-57; M-1 to Q-56; M-1 to W-55; M-1 to A-54; M-1 to K-53; M-1 to Q-52; M-1 to S-51; M-1 to S-50; M-1 to A-49; M-1 to F-48; M-1 to K-47; M-1 to E-46; M-1 to L-45; M-1 to T-44; M-1 to Y-43; M-1 to D-42; M-1 to C-41; M-1 to K-40; M-1 to V-39; M-1 to D-38; M-1 to L-37; M-1 to T-36; M-1 to Q-35; M-1 to G-34; M-1 to E-33; M-1 to K-32; M-1 to L-31; M-1 to E-30; M-1 to Y-29; M-1 to K-28; M-1 to E-27; M-1 to E-

09881 06130 T 228860

26; M-1 to T-25; M-1 to L-24; M-1 to K-23; M-1 to T-22; M-1 to A-21; M-1 to A-20; M-1 to R-19; M-1 to L-18; M-1 to E-17; M-1 to S-16; M-1 to V-15; M-1 to F-14; M-1 to L-13; M-1 to M-12; M-1 to W-11; M-1 to L-10; M-1 to L-9; M-1 to G-8; and/or M-1 to W-7; of SEQ ID NO: 478. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to inhibit the Mixed Lymphocyte Reaction), other functional activities (e.g., biological activities, ability to multimerize, ability to bind ligand, ability to generate antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response. Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the polypeptide shown in Figures 1A-B (SEQ ID NO: 478), as described by the general formula 1-n, where n is an integer from 6 to 228, where n corresponds to the position of the amino acid residue identified in SEQ ID NO: 478.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the mature extracellular portion of the B7-H5 protein (SEQ ID NO: 760): K-28 to I-198; Y-29 to I-198; E-30 to I-198; L-31 to I-198; K-32 to I-198; E-33 to I-198; G-34 to I-198; Q-35 to I-198; T-36 to I-198; L-37 to I-198; D-38 to I-198; V-39 to I-198; K-40 to I-198; C-41 to I-198; D-42 to I-198; Y-43 to I-198; T-44 to I-198; L-45 to I-198; E-46 to I-198; K-47 to I-198; F-

48 to I-198; A-49 to I-198; S-50 to I-198; S-51 to I-198; Q-52 to I-198; K-53 to I-198; A-54 to I-198; W-55 to I-198; Q-56 to I-198; I-57 to I-198; I-58 to I-198; R-59 to I-198; D-60 to I-198; G-61 to I-198; E-62 to I-198; M-63 to I-198; P-64 to I-198; K-65 to I-198; T-66 to I-198; L-67 to I-198; A-68 to I-198; C-69 to I-198; T-70 to I-198; E-71 to I-198; R-72 to I-198; P-73 to I-198; S-74 to I-198; K-75 to I-198; N-76 to I-198; S-77 to I-198; H-78 to I-198; P-79 to I-198; V-80 to I-198; Q-81 to I-198; V-82 to I-198; G-83 to I-198; R-84 to I-198; I-85 to I-198; I-86 to I-198; L-87 to I-198; E-88 to I-198; D-89 to I-198; Y-90 to I-198; H-91 to I-198; D-92 to I-198; H-93 to I-198; G-94 to I-198; L-95 to I-198; L-96 to I-198; R-97 to I-198; V-98 to I-198; R-99 to I-198; M-100 to I-198; V-101 to I-198; N-102 to I-198; L-103 to I-198; Q-104 to I-198; V-105 to I-198; E-106 to I-198; D-107 to I-198; S-108 to I-198; G-109 to I-198; L-110 to I-198; Y-111 to I-198; Q-112 to I-198; C-113 to I-198; V-114 to I-198; I-115 to I-198; Y-116 to I-198; Q-117 to I-198; P-118 to I-198; P-119 to I-198; K-120 to I-198; E-121 to I-198; P-122 to I-198; H-123 to I-198; M-124 to I-198; L-125 to I-198; F-126 to I-198; D-127 to I-198; R-128 to I-198; I-129 to I-198; R-130 to I-198; L-131 to I-198; V-132 to I-198; V-133 to I-198; T-134 to I-198; K-135 to I-198; G-136 to I-198; F-137 to I-198; S-138 to I-198; G-139 to I-198; T-140 to I-198; P-141 to I-198; G-142 to I-198; S-143 to I-198; N-144 to I-198; E-145 to I-198; N-146 to I-198; S-147 to I-198; T-148 to I-198; Q-149 to I-198; N-150 to I-198; V-151 to I-198; Y-152 to I-198; K-153 to I-198; I-154 to I-198; P-155 to I-198; P-156 to I-198; T-157 to I-198; T-158 to I-198; T-159 to I-198; K-160 to I-198; A-161 to I-198; L-162 to I-198; C-163 to I-198; P-164 to I-198; L-165 to I-198; Y-166 to I-198; T-167 to I-198; S-168 to I-198; P-169 to I-198; R-170 to I-198; T-171 to I-198; V-172 to I-198; T-173 to I-198; Q-174 to I-198; A-175 to I-198; P-176 to I-198; P-177 to I-198; K-178 to I-198; S-179 to I-198; T-180 to I-198; A-181 to I-198; D-182 to I-198; V-183 to I-198; S-184 to I-198; T-185 to I-198; P-186 to I-198; D-187 to I-198; S-188 to I-198; E-189 to I-198; I-190 to I-198; N-191 to I-198; L-192 to I-198; T-193 to I-198; and/or K-23 to A-29 of SEQ ID NO: 478. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the mature extracellular portion of the B7-H5 protein

(SEQ ID NO: 760): E-27 to D-197; E-27 to T-196; E-27 to V-195; E-27 to N-194; E-27 to T-193; E-27 to L-192; E-27 to N-191; E-27 to I-190; E-27 to E-189; E-27 to S-188; E-27 to D-187; E-27 to P-186; E-27 to T-185; E-27 to S-184; E-27 to V-183; E-27 to D-182; E-27 to A-181; E-27 to T-180; E-27 to S-179; E-27 to K-178; E-27 to P-177; E-27 to P-176; E-27 to A-175; E-27 to Q-174; E-27 to T-173; E-27 to V-172; E-27 to T-171; E-27 to R-170; E-27 to P-169; E-27 to S-168; E-27 to T-167; E-27 to Y-166; E-27 to L-165; E-27 to P-164; E-27 to C-163; E-27 to L-162; E-27 to A-161; E-27 to K-160; E-27 to T-159; E-27 to T-158; E-27 to T-157; E-27 to P-156; E-27 to P-155; E-27 to I-154; E-27 to K-153; E-27 to Y-152; E-27 to V-151; E-27 to N-150; E-27 to Q-149; E-27 to T-148; E-27 to S-147; E-27 to N-146; E-27 to E-145; E-27 to N-144; E-27 to S-143; E-27 to G-142; E-27 to P-141; E-27 to T-140; E-27 to G-139; E-27 to S-138; E-27 to F-137; E-27 to G-136; E-27 to K-135; E-27 to T-134; E-27 to V-133; E-27 to V-132; E-27 to L-131; E-27 to R-130; E-27 to I-129; E-27 to R-128; E-27 to D-127; E-27 to F-126; E-27 to L-125; E-27 to M-124; E-27 to H-123; E-27 to P-122; E-27 to E-121; E-27 to K-120; E-27 to P-119; E-27 to P-118; E-27 to Q-117; E-27 to Y-116; E-27 to I-115; E-27 to V-114; E-27 to C-113; E-27 to Q-112; E-27 to Y-111; E-27 to L-110; E-27 to G-109; E-27 to S-108; E-27 to D-107; E-27 to E-106; E-27 to V-105; E-27 to Q-104; E-27 to L-103; E-27 to N-102; E-27 to V-101; E-27 to M-100; E-27 to R-99; E-27 to V-98; E-27 to R-97; E-27 to L-96; E-27 to L-95; E-27 to G-94; E-27 to H-93; E-27 to D-92; E-27 to H-91; E-27 to Y-90; E-27 to D-89; E-27 to E-88; E-27 to L-87; E-27 to I-86; E-27 to I-85; E-27 to R-84; E-27 to G-83; E-27 to V-82; E-27 to Q-81; E-27 to V-80; E-27 to P-79; E-27 to H-78; E-27 to S-77; E-27 to N-76; E-27 to K-75; E-27 to S-74; E-27 to P-73; E-27 to R-72; E-27 to E-71; E-27 to T-70; E-27 to C-69; E-27 to A-68; E-27 to L-67; E-27 to T-66; E-27 to K-65; E-27 to P-64; E-27 to M-63; E-27 to E-62; E-27 to G-61; E-27 to D-60; E-27 to R-59; E-27 to I-58; E-27 to I-57; E-27 to Q-56; E-27 to W-55; E-27 to A-54; E-27 to K-53; E-27 to Q-52; E-27 to S-51; E-27 to S-50; E-27 to A-49; E-27 to F-48; E-27 to K-47; E-27 to E-46; E-27 to L-45; E-27 to T-44; E-27 to Y-43; E-27 to D-42; E-27 to C-41; E-27 to K-40; E-27 to V-39; E-27 to D-38; E-27 to L-37; E-27 to T-36; E-27 to Q-35; E-27 to G-34; and/or E-27 to E-33 of SEQ ID NO: 478. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

ORIGINAL PAGE

In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 478, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. 97903 and/or 209049, where this portion excludes any integer of amino acid residues from 1 to about 228 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. 97903 and/or 209049, or any integer of amino acid residues from 1 to about 228 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. 97903 and/or 209049. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of immune system tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases and/or disorders involving immune system activation,

stimulation and/or surveillance, particularly involving T cells and/or neutrophils. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). Particularly contemplated are the use of antibodies directed against the

5 extracellular portion of this protein which act as antagonists for the activity of the B7-H5 protein. Such antagonistic antibodies would be useful for the prevention and/or inhibition of such biological activities as are disclosed herein (e.g. T cell modulated activities).

For a number of disorders of the above tissues or cells, particularly of the

10 immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in

15 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune cells (e.g., T-cells, neutrophils), and the homology to members of the B7 family of ligands, indicates that the polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders involving immune system activation,

20 stimulation and/or surveillance, particularly as relating to T cells and/or neutrophils. In particular, the translation product of the B7-H5 gene may be involved in the costimulation of T cells, binding to ICOS, and/or may play a role in modulation of the expression of particular cytokines. Representative uses are also described in the "Immune Activity" section below and elsewhere herein.

More generally, the tissue distribution in immun system cells indicates that this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the

30 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS,

05682471.051801

leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of

5 various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents
10 that modulate their interactions, in addition to its use as a nutritional supplement.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 169 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
15 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 888 of SEQ ID NO: 169, b is an integer of 15 to 902, where both a and b correspond to the
20 positions of nucleotide residues shown in SEQ ID NO: 169, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 160

The translation product of this gene shares sequence homology with mouse X
25 inactive specific transcript protein which is thought to be important in X chromosome inactivation.

This gene is expressed primarily in HSA172 cell and to a lesser extent in normal ovary tissue, ovarian cancer, frontal cortex and brain.

Therefore, polynucleotides and polypeptides of the invention are useful as
30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: ovarian tumor, schizophrenia and other neurological disorders. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural system, expression of this gene at significantly higher or lower levels may be routinely
 5 detected in certain tissues and cell types (e.g., ovary and other reproductive tissue, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily
 10 fluid from an individual not having the disorder.

The tissue distribution and homology to X inactive specific transcript protein indicate that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of reproductive system tumors and CNS tumors.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
 15 479 as residues: His-48 to Trp-56.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:170 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
 20 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1869 of SEQ ID NO:170, b is an integer of 15 to 1883, where both a and b correspond to the positions of
 25 nucleotide residues shown in SEQ ID NO:170, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 161

This gene is expressed primarily in adipose cells and to a lesser extent in liver
 30 and prostate tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

0988217.061804
 FOR 90 T 278860

biological sample and for diagnosis of diseases and conditions: obesity and liver disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the adipose cell, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., adipose cells, liver, and prostate, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment of obesity and liver disorder.

This gene is believed to reside on chromosome 3. Polynucleotides related to this gene are useful, therefore, in linkage analysis for chromosome 3.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:171 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2086 of SEQ ID NO:171, b is an integer of 15 to 2100, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:171, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 162

The translation product of this gene shares sequence homology with yeast ubiquitin activating enzyme homolog which is thought to be important in protein posttraslation processing. Mammalian cells contain two distinct proteolytic pathways that are involved in different aspects of protein breakdown. One of these is ubiquitin-

dependent, it is a major pathway in eukaryotes involved in the selective degradation of abnormal and short-lived proteins. Ubiquitin is a highly conserved 76 amino acid residue protein present in eukaryotic cells either free or covalently attached to a great variety of proteins.

5 The ubiquitin gene is one of the genes known to be stimulated during the apoptotic death program and ubiquitin of nuclear proteins might be involved in chromatin disorganization and oligonucleosomal fragmentation, which are among the key events occurring in apoptosis. Apoptosis, the classical type of programmed cell death, can be triggered in many cell types by widely diverse stimuli, for example,
10 gamma rays at low doses can induce apoptosis in vitro in interphase human lymphocytes. In this type of apoptosis induction, activated gene expression is necessary for the fulfillment of the death program. It has been reported (Delic, J., et al., *Mol. Cell Biol.*, 13:4875, 83 (1993)) that there is a relationship between ubiquitin gene expression or ubiquitination and gamma-irradiation-mediated apoptosis in
15 normal circulating human lymphocytes. In this report it has been demonstrated that the ubiquitin mRNA level is increased as a consequence of the activation of ubiquitin gene transcription 15 to 90 minutes after initiation of apoptosis; specifically, in apoptotic cells, and not in all irradiated cells, nuclear proteins are highly ubiquitinated; and ubiquitin sequence-specific antisense oligonucleotide inhibition
20 results in a decreased level of ubiquitinated nuclear proteins and considerably diminishes the proportion of cells exhibiting the apoptotic death pattern.

Perturbations of ubiquitin system can also induce a programmed necrotic response in plants such as leaf curling, vascular tissue alterations and necrotic lesions.

Ubiquitin can inhibit the cytotoxic properties of platelets and the production of
25 oxygen metabolites by these cells. Moreover, this molecule is able to act as a proaggregating factor and seems of a great interest in pathologies involving defects in platelet aggregation. Ubiquitin also plays a role in the regulation of immunological disorders in which platelets seem to be implicated such as hymenoptera venom hypersensitivity and aspirin-sensitive asthma, since in both situations, ubiquitin is
30 able to inhibit the cytotoxic function of platelets. Ubiquitin has also been shown to be increased in patients with Alzheimer's disease (Taddei, N., et al., *Neurosci. Lett.*, 151:158-61 (1993)). This study concerned the amount of soluble ubiquitin in

different cortical and subcortical regions of brains from patients with Alzheimer's disease compared to the amount in a normal brain. The soluble ubiquitin content was significantly higher in pathological tissue than in normal tissue. The primary structure of ubiquitin isolated from brain tissue affected by Alzheimer's degenerative processes was determined and resulted to be identical to normal human ubiquitin. This report suggests that an impairment of the process of intracellular, ubiquitin-dependent proteolysis might play an important role in the pathogenesis of this neurodegenerative disease.

Ubiquitin-proteasome system also plays a major role in specific processing and subsequent presentation of MHC class I-restricted antigens.

Maturation of the p105 NF- κ B precursor into the active p50 subunit of the transcriptional activator also proceeds in a ubiquitin and proteasome-dependent manner. Furthermore, inhibitors to the proteasome block degradation of I κ B α and thus prevent tumor necrosis factor alpha induced activation of NF- κ B and its entry into the nucleus.

The unstable c-Jun, but not the stable v-Jun, is multi-ubiquitinated and degraded. The escape of the oncogenic v-Jun from ubiquitin-dependent degradation suggests a route to the malignant transformation. Another proto-oncoprotein, c-Mos, is also degraded by the ubiquitin system.

The human papilloma virus (HPV) derived E6 proteins stimulate ATP and ubiquitin dependent conjugation and degradation of p53, such a mechanism could explain the extremely low levels of p53 observed in HPV-transformed cervical carcinoma lines and propose a mechanism for the tumorigenicity of these oncoproteins.

Several cell surface receptors, including the lymphocyte homing receptor, growth hormone receptor, and growth factor receptor (PDGF, steel factor) were also found to be modified by ubiquitin.

This gene is expressed primarily in stromal cell and to a lesser extent in retina, human atrophic endometrium, colon carcinoma and myeloid progenitor cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: defects of stromal cell

09882471.051801

development, neuronal growth disorders and tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., retinal cells, endometrium, colon, and bone marrow, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to ubiquitin-activating enzyme homolog indicate that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis or treatment of some type of tumors, fucosidosis and neuronal growth disorders.

Once the polypeptides are being expressed intra-cellularly via gene therapy, they may be employed to provide a signal for the lymphocyte homing receptor thereby regulating lymphocyte trafficking. The growth hormone receptor also utilizes ubiquitin to signal ligands, and, therefore, the Ubiquitin Activating-like polypeptides may be employed to regulate activation of the growth receptor.

Ubiquitin Activating-like polypeptides may be employed to overcome many viral infections by overcoming the suppressed programmed cell death induced by these viruses, since programmed cell death may be one of the primary antiviral defense mechanisms of cells.

Ubiquitin Activating-like polypeptides may also be employed to treat immuno-suppression related disorders, such as AIDS, by targeting virus infected cells for cell death.

Ubiquitin Activating-like polypeptides may also be employed to inhibit the cytotoxic properties of platelets and the production of oxygen metabolites by platelets. These polypeptides may also be employed to regulate immunological disorders in which platelets seem to be involved, for example, hymenoptera venom hypersensitivity and aspirin-sensitive asthma.

Ubiquitin Activating-like polypeptides may also be employed to treat malignant transformation because proto-oncoproteins c-Mos and v-Jun are degraded in a ubiquitin-dependent manner.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 481 as residues: Lys-65 to Lys-72, Tyr-159 to Thr-169, Arg-176 to Pro-182, Glu-200 to Glu-214, Ala-228 to Asp-234, Ile-238 to Asp-250, Lys-271 to Pro-279, Val-287 to Gly-306, Asp-345 to Ser-350, Asn-419 to Lys-426, Glu-494 to Leu-506.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:172 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1916 of SEQ ID NO:172, b is an integer of 15 to 1930, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:172, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 163

This gene is expressed primarily in primary breast cancer and hemangiopericytoma and to a lesser extent in adult brain and cerebellum.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: breast cancer, leukemia and cerebellum disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., mammary tissue, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma,

urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis or treatment of various tumors and disease involved in neural system.

10 This gene is believed to reside on chromosome 3. Polynucleotides related to this gene are believed to be useful, therefore, in linkage analysis as markers for chromosome 3.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 482 as residues: Glu-29 to Tyr-42, Glu-44 to Glu-54, Tyr-68 to Glu-73, Ala-145 to Leu-165, Gln-173 to Ser-183, Lys-215 to Cys-220.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:173 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
20 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1495 of SEQ ID NO:173, b is an integer of 15 to 1509, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:173, and where the b is greater than or equal to a + 14.

25

FEATURES OF PROTEIN ENCODED BY GENE NO: 164

30 The translation product of this gene, "MAX.3", shares sequence homology with proline rich proteins. Recently, another group has also cloned this gene, calling it CD84 leukocyte antigen, a new member of the Immunoglobulin superfamily. MAX.3 detects the CD84 antigen, which is a member of the CD2 family of cell surface molecules, and it is postulated that it is this interaction which allows MAX.3 to function in intracellular signaling events. (See Accession No. U82988, see also,

Blood 90 (6), 2398-2405 (1997), incorporated herein by reference.) The extracellular domain is believed to comprise residues 23-220, the transmembrane domain residues 221-245 and the intracellular domain residues 246-389, of SEQ ID NO:483.

Preferred polypeptides of the invention comprise the extracellular domain alone,
 5 residues 23-220, or fused to the intracellular domain; i.e., lacking the transmembrane domain.

This gene is expressed primarily in Weizmann olfactory tissue and osteoclastoma and to a lesser extent in anergic T-cells.

Therefore, polynucleotides and polypeptides of the invention are useful as
 10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: osteoporosis and immune disease. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,
 15 particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., olfactory tissue, bone, and blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene
 20 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to the Ig superfamily CD84 antigen indicate that the protein product of this clone is useful for treatment of osteoporosis, autoimmune disease, and other immune disorders. Alternatively, the tissue
 25 distribution and homology to a cell-surface antigen may indicate that MAX.3 plays a role in the extracellular matrix remodelling and/or wound healing immune responses in the body.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 483 as residues: Ala-19 to Ser-24, Glu-102 to Ile-111, Thr-113 to Tyr-124, Glu-158 to
 30 Asn-167, Thr-183 to Leu-189, Asn-197 to Ser-207, Lys-250 to Thr-260, Arg-269 to Ile-278, Pro-289 to Pro-294, Ala-311 to Ser-323.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:174 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3159 of SEQ ID NO:174, b is an integer of 15 to 3173, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:174, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 166

This gene is expressed primarily in human primary breast cancer and to a lesser extent in activated monocyte. This polypeptide is a type Ia transmembrane protein. The extracellular domain is believed to comprise residues from about 20-195, the transmembrane domain residues from about 196 to about 212, and the cytoplasmic tail residues from about 213 to about 217, of SEQ ID NO: 485. Preferred polypeptide fragments comprise the amino acid sequence:

VTQPKHLSASMGGSEIPFSFYYPWELAXXPXVRISWRRGHFHG
QSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQSVYFCRVELDT
RRSG (SEQ ID NO: 754), as well as N-terminal and C-terminal deletions. Also preferred are polypeptides comprising the extracellular domain, residues 20 to 195. Also provided are polynucleotide fragments encoding these polypeptides.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., mammary tissue, and blood

09882171.061801

cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis of breast cancer and other immune system disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:176 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:176, b is an integer of 15 to 1290, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:176, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 167

This gene is expressed primarily in fetal tissues and to a lesser extent in adult lung. This gene has also been mapped to chromosomal location 9q34, and thus, can be used as a marker for linkage analysis for chromosome 9.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the embryo tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., fetal tissues, and lung, and cancerous and wounded tissues) or bodily

fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful as reagents for differential identification of tissues or cell types present in a biological sample.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 486 as residues: Tyr-15 to Gly-23.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:177 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2276 of SEQ ID NO:177, b is an integer of 15 to 2290, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:177, and where the b is greater than or
20 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 168

The translation product of this gene shares sequence homology with Ig Heavy Chain which is thought to be important in immune response.

25 This gene is expressed primarily in prostate cancer tissue specifically

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: prostate cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in
30 providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues and cell types (e.g., prostate, tissue and cells of the immune system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis of prostate cancer and other immune system disorders.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:178 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 535 of SEQ ID NO:178, b is an integer of 15 to 549, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:178, and where the b is greater than or
20 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 169

25 The translation product of this gene shares sequence homology with cytosolic acyl coenzyme-A hydrolase, which is thought to be important in neuron-specific fatty acid metabolism. The gene represented by this contig has since been published by Hajra and colleagues (GenBank Accession No. U91316, incorporated herein by reference).

30 This gene is expressed primarily in human pituitary gland and to a lesser extent in colorectal cancer tissue. This gene has also been observed in the LNCAP cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions: hyperlipidemias of familial and/or idiopathic origins. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above

5 tissues or cells, particularly blood, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., pituitary and colon, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

10 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to rat cytosolic acyl coenzyme-A hydrolase indicate that polynucleotides and polypeptides corresponding to this gene are useful for the detection or treatment of hyperlipidemia disease states by virtue of

15 the ability of specific drugs to activate the enzyme.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 488 as residues: Arg-48 to Glu-56.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

20 related to SEQ ID NO:179 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

25 general formula of a-b, where a is any integer between 1 to 1495 of SEQ ID NO:179, b is an integer of 15 to 1509, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:179, and where the b is greater than or equal to a + 14.

30 FEATURES OF PROTEIN ENCODED BY GENE NO: 170

The translation product of this gene shares sequence homology with a *Caenorhabditis elegans* gene which is thought to be important in organism development. This gene is believed to be important in embryogenesis and cancer.

Preferred polypeptide encoded by this gene comprise the following amino acid sequence:

MATPAVPVSAPPATPTVPAAAPASVPAPTPAPAAAPVPAAAPACILRPC
GSSGCNCGSWDPGLSAXPAQTPAPALPGPALPGPFPGGRVVRLHPVILAIVD
SY
ERRNEGAARVIGTLLGTVDKHSVEVTNCFSPHNESEDEVAVDMEFAKNMY
ELHKKVSPNELILGWYATGHDITEHSVLIHEYYSREAPNPIHLTVDTSLQNGR
MSIKAYVSTLMGVPGRTMGVMFTPLTVKYAAYDTERIGVDLIMKTCFSPNRV
IGLSSDLQQVGGASARIQDALSTVLQYAEDVLSGKVSADNTVGRFLMSLVNQ
VPKIVPDDFETMLNSNINDLLMVTYLANLTQSQIALNEKLVNL (SEQ ID NO:
755) and MLNSNINDLLMVTYLANLTQSQIALNEKLVNL (SEQ ID NO: 756).

Polynucleotides encoding such polypeptides are also provided.

This gene is expressed primarily in human synovial sarcoma tissue, bone marrow, and to a lesser extent in human brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, bone, specifically synovial sarcoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the bone, connective tissues and possibly immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., synovial tissue, bone marrow, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to *Caenorhabditis elegans* indicate that polynucleotides and polypeptides corresponding to this gene are useful as a diagnostic and/or therapeutic modality directed at the detection and/or treatment of connective tissue sarcomas or other related bone diseases.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:180 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1302 of SEQ ID NO:180, b is an integer of 15 to 1316, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:180, and where the b is greater than or
15 equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 171

The translation product of this gene shares sequence homology with beta1-6GlcNAc transferase which is thought to be important in the transfer and metabolism
20 of beta1-6, N-acetylglucosamine. This gene product has previously been shown to suppress melanoma lung metastasis in both syngeneic and nude mice, decreased invasiveness into the matrigel, and inhibition of cell attachment to collagen and laminin without affecting cell growth.

This gene is expressed primarily in human testes and prostate tissues, and to a
25 lesser extent in kidney, medulla, and pancreas.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer particularly melanoma. Similarly, polypeptides and antibodies
30 directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene

at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testes and other reproductive tissue, prostate, kidney, pancreas, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to beta1-6GlcNAc transferase indicate that the protein product of this clone is useful for the development of diagnostic and/or therapeutic modalities directed at the detection and/or treatment of cancer, the metastasis of malignant tissue or cells. Defects in this potentially secreted enzyme may play a role in metastasis.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:181 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 763 of SEQ ID NO:181, b is an integer of 15 to 777, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:181, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 172

This gene is expressed primarily in fetal spleen and liver.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: immune disorders, Wilm's tumor disease, hepatic disorders, and hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For

a number of disorders of the above tissues or cells, particularly of the hematopoiesis and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., spleen and liver, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and identification of fetal defects along with correcting diseases that affect hematopoiesis and the immune system.

This gene is believed to reside on chromosome 6. Therefore, nucleic acids related to this gene are useful in linkage analysis as markers for chromosome 6.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:182 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 777 of SEQ ID NO:182, b is an integer of 15 to 791, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:182, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 173

The translation product of this gene shares sequence homology with ret II oncogene which is thought to be important in Hirschsprung disease and many types of cancers.

This gene is expressed in multiple tissues including the lymphatic system, brain, and thyroid.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: Hirschsprung disease and multiple cancers. Similarly, polypeptides and antibodies directed to these polypeptides are
 5 useful in providing immunological probes for identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., lymphoid tissue, thyroid, and brain and other tissue of the nervous system, and cancerous and
 10 wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to ret II oncogene indicate that
 15 polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of various cancers. It would also be useful for the diagnosis and treatment of Hirschsprung disease. Preferred polypeptides of the invention comprise the amino acid sequence:

MEAQQVNEAESAREQLQXLHDQIAGQKASKQELETELERLKQEFHYIEE
 20 DLYRTKNTLQSRIKDRDEEIQKLRNQLTNKTLSSSQSELENRLHQLTETLIQK
 QTMLESLSSTEKNSLVFQLERLEQQMNSASGSSSNGSSINMSGIDNGEGTRLRN
 VPVLFNDTETNLAGMYGKVRKAASSIDQFSIRLGIFLRRYPIARVFVIIYMALL
 HLWVMIVLLTYTPMHHDQPYGK (SEQ ID NO: 757). Polynucleotides
 encoding such polypeptides are also provided.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:183 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
 30 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1391 of SEQ ID NO:183,

0988271.061801

b is an integer of 15 to 1405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:183, and where the b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 174

The translation product of this gene shares sequence homology with testis enhanced gene transcript which is thought to be important in regulation of human development.

10 This gene is expressed primarily in infant brain and to a lesser extent in a variety of other tissues and cell types, including the prostate, testes, monocytes, macrophages, dendritic cells, keratinocytes, and adipocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neurological,
 15 developmental, immune and inflammation disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and immune systems, expression of this gene at significantly higher or lower levels may be routinely
 20 detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, prostate, testes and other reproductive tissue, blood cells, keratinocytes, and adipocytes, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,
 25 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to testis enhanced gene transcript indicate that the protein product of this clone is useful for diagnosis and treatment of disorders involving the developing brain and the immune system.

30 This gene is believed to reside on chromosome 10. Nucleic acids related to this gene are useful therefore in linkage analysis as markers for chromosome 10.

T03190" 1-22886

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 493 as residues: Ser-31 to Trp-37, Pro-41 to Thr-49, Arg-54 to Lys-63, Tyr-119 to His-125, Pro-181 to Lys-189, Gly-340 to Lys-345.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:184 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1582 of SEQ ID NO:184, b is an integer of 15 to 1596, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:184, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 175

This gene is expressed primarily in prostate and to a lesser extent in various other tissues, including placenta.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancers, especially of the prostate. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., prostate and placenta, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this clone is useful for diagnosis and treatment of prostate disorders and cancer. It may also be useful for the diagnosis and treatment of endocrine disorders.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 494 as residues: Lys-10 to Trp-19, Arg-49 to Leu-54, Val-78 to Val-87, Pro-141 to Lys-148.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:185 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2279 of SEQ ID NO:185, b is an integer of 15 to 2293, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:185, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 176

The translation product of this gene shares sequence homology with *Sacchromyces cerevisiae* YNT20 gene which is thought to be important in mitochondrial function.

This gene is expressed at a particularly high level in muscle tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases related to such tissues and cell types including: muscle wasting diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neuromuscular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., muscle and cancerous and wounded-tissues) or bodily fluids (e.g.,

serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 The tissue distribution and homology to the YNT20 gene indicate that this protein is useful for treatment and detection of neuromuscular diseases caused by loss of mitochondrial function. For example this gene or its protein product could be used in replacement therapy for such diseases.

10 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 495 as residues: Arg-91 to Pro-96, Val-106 to Leu-113, Lys-180 to Lys-187, Asn-191 to Val-198.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:186 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1198 of SEQ ID NO:186,
20 b is an integer of 15 to 1212, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:186, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 177

25 This gene is expressed primarily in the brain and to a lesser extent in kidney, placenta, smooth muscle, heart and lung.

 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: neuromuscular
30 diseases, degenerative diseases of the central nervous system, and heart disease. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the neuromuscular system, central nervous system, and heart, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, kidney, placenta, muscle, heart and lung, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

This gene or its protein product could also be used for replacement therapy for the above mentioned diseases.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 496 as residues: Arg-12 to Glu-18, Asn-38 to Phe-47, Arg-56 to Val-62.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:187 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1591 of SEQ ID NO:187, b is an integer of 15 to 1605, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:187, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 178

The translation product of this gene shares sequence homology with caldesmon which is thought to be important in the cellular response to changes in glucose levels.

This gene is expressed primarily in multiple tissues including brain and retina.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions: central nervous system disorders and retinopathy. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS disorders and retinopathy, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, and retinal tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to caldesmon indicate that polynucleotides and polypeptides corresponding to this gene are useful for treatment of retinopathies.

This gene is believed to be useful as a marker for chromosomes 1 and 3 in linkage analysis studies.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 497 as residues: Met-1 to Gly-7, Glu-17 to Glu-28, Lys-39 to Asp-45, Ser-50 to Ser-63, Glu-82 to Phe-88, Pro-97 to Lys-109, Gln-124 to Arg-129, Val-145 to Lys-157, Glu-163 to Leu-168, Ile-186 to Glu-195, Glu-198 to Arg-230, Asn-240 to Phe-252, Pro-256 to Glu-264.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:188 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1502 of SEQ ID NO:188, b is an integer of 15 to 1516, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:188, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 179

The translation product of this gene shares sequence homology with mouse fibrosin protein which is thought to be important in regulation of fibrinogenesis in certain chronic inflammatory diseases.

This gene is expressed primarily in amniotic cells and breast tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of breast cancer and abnormal embryo development. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., amniotic cells, and mammary tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to fibrosin indicate that the protein product of this clone is useful for treatment of breast cancer. This gene or its protein product could be used in replacement therapy for breast cancer. In addition the protein product of this gene is useful in the treatment of chronic inflammatory diseases.

This gene is believed to reside on chromosome 15. Polynucleotides related to this gene are believed to be useful, therefore, in linkage analysis as markers for chromosome 15.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:189 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 667 of SEQ ID NO:189, b is an integer of 15 to 681, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:189, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 180

This gene is expressed in several infant tissues including brain and liver and various adult tissues including brain, lung, liver, testes, and prostate.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, brain cancer, lung cancer, liver cancer and cancers of the reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, hepatic system, and reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, lung, liver, testes and other reproductive tissue, and prostate, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene product indicates that the protein product of this clone is involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 499 as residues: Pro-153 to Arg-158.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:190 and may have been publicly available prior to conception

of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
 5 general formula of a-b, where a is any integer between 1 to 1000 of SEQ ID NO:190, b is an integer of 15 to 1014, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:190, and where the b is greater than or equal to a + 14.

10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 181**

This gene is expressed primarily in activated monocytes and to a lesser extent in melanocytes and dendritic cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a
 15 biological sample and for diagnosis of immune system diseases and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely
 20 detected in certain tissues and cell types (e.g., blood cells, melanocytes, and dendritic cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the
 25 disorder.

The tissue distribution indicates that the protein product of this clone could be involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
 30 500 as residues: Asp-35 to Asp-41, Ser-72 to Glu-77, Lys-110 to His-115, Pro-160 to Gln-165.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:191 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2765 of SEQ ID NO:191, b is an integer of 15 to 2779, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:191, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 182

This gene is expressed primarily in placenta and several tumors of various tissue origin and to a lesser extent in normal tissues including liver, lung, brain, and skin,

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of cancers of all kinds. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, respiratory system and skin, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., liver, lung, brain and other tissues of the nervous system, and skin, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The high expression of this gene in multiple tumors indicates that the protein product of the clone may be involved in cell growth control and therefore would be useful for treatment of certain cancers. Likewise molecules developed to block the

09882171.061801

activity of the protein product of this clone could be used to block its potential role in tumor growth promotion.

This gene is believed to reside on chromosome 6. Polynucleotides related to this gene are believed, therefore, to be useful in linkage analysis for chromosome 6.

5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 501 as residues: Gln-37 to Gln-43, Cys-51 to Cys-65.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:192 and may have been publicly available prior to conception
10 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1909 of SEQ ID NO:192,
15 b is an integer of 15 to 1923, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:192, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 183

20 The translation product of this gene shares sequence homology with the mouse Ndr1 gene which is thought to be important in cancer progression and atherogenesis (see J Biol Chem. 1996 Nov 22; 271(47): 29659-29665), incorporated herein by reference in its entirety.

This gene is expressed in multiple cell types and tissues including brain, lung,
25 kidney, bone marrow, liver, and spleen.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of all types of cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological
30 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous, immune, and endocrine systems, expression of this gene at significantly higher or lower levels may

09882171.061801

be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, lung, kidney, bone marrow, liver and spleen, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a
 5 disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to Ndr1 gene, which is thought to be involved in cancer progression, indicate that polynucleotides and polypeptides corresponding to this gene are useful for treatment of certain cancers. Likewise
 10 molecules developed to block the activity of the protein product of this clone could be used to block its potential role in tumor growth promotion.

This gene is believed to reside on chromosome 8. Polynucleotides derived from this gene are useful, therefore, in linkage analysis as markers for chromosome 8.

Many polynucleotide sequences, such as EST sequences, are publicly
 15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:193 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
 20 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2332 of SEQ ID NO:193, b is an integer of 15 to 2346, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:193, and where the b is greater than or equal to a + 14.

25 **FEATURES OF PROTEIN ENCODED BY GENE NO: 184**

This gene is expressed primarily in early stage human brain and liver and to a lesser extent in several other fetal tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as
 30 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions: brain and liver cancers. Similarly, polypeptides and antibodies directed to these polypeptides are

T09T90" T2T22222

useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, liver, and fetal tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression of this gene in embryonic tissues indicates that the protein could be involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

This gene is believed to reside on chromosome 11. Polynucleotides derived from this gene are useful, therefore, in linkage analysis as markers for chromosome 11.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 503 as residues: Pro-116 to Ser-127, Pro-136 to Tyr-146.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:194 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3040 of SEQ ID NO:194, b is an integer of 15 to 3054, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:194, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 185

This gene is expressed primarily in infant and embryonic brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of degenerative nervous system disorders and brain cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., embryonic tissue, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression of this gene in embryonic tissues indicates that the protein could be involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:195 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 893 of SEQ ID NO:195, b is an integer of 15 to 907, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:195, and where the b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 186

This gene is expressed primarily in multiple tissues including placenta, fetal lung, fetal liver, and brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of all types of cancers including liver, brain and lung. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, pulmonary system, and hepatic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, lung, liver, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression of this gene in embryonic tissues indicates that the protein could be involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

This gene is believed to reside on chromosome 10. Polynucleotides derived from this gene are useful, therefore, in linkage analysis as markers for chromosome 10.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 505 as residues: Pro-10 to Glu-17, Gly-42 to Gln-51.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:196 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:196, b is an integer of 15 to 1290, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:196, and where the b is greater than or equal to a + 14.

09882171.061801
T08T90" T/28860

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of all types of cancers including liver, brain and lung. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, pulmonary system, and hepatic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., placenta, lung, liver, and brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression of this gene in embryonic tissues indicates that the protein could be involved in growth regulation and could be used as a growth factor or growth blocker in a variety of settings including treatment of cancers.

This gene is believed to reside on chromosome 10. Polynucleotides derived from this gene are useful, therefore, in linkage analysis as markers for chromosome 10.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 505 as residues: Pro-10 to Glu-17, Gly-42 to Gln-51.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:196 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:196, b is an integer of 15 to 1290, where both a and b correspond to the positions of

Table 1

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HTTEZ21	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	11	1019	262	859	177	177	320	1	17	18	22
1	HTTEZ21	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	197	582	1	582	177	177	506	1	17	18	22
1	HTTEZ21	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	198	1020	296	830	442	442	507	1	18	19	22
2	HBCBW52	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	12	465	1	465	81	81	321	1	30	31	128
2	HBCBW52	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	199	524	229	343		196	508	1	20	21	33
3	HCUFM41	97897 02/26/97 209043 05/15/97	ZAP Express	13	474	1	474	1	1	322	1	24	25	28
3	HCUFM41	97897 02/26/97 209043 05/15/97	ZAP Express	200	332	1	319	35	35	509	1	24	25	28

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
4	HCUFQ22	97897 02/26/97 209043 05/15/97	ZAP Express	14	314	1	298	122	122	323	1	34	35	64
5	HCUFV01	97897 02/26/97 209043 05/15/97	ZAP Express	15	613	1	613	30	30	324	1	18	19	21
6	HCUGA50	97897 02/26/97 209043 05/15/97	ZAP Express	16	356	1	356	239	239	325	1	22	23	39
7	HCUIM14	97897 02/26/97 209043 05/15/97	ZAP Express	17	414	185	414	278	278	326	1	26	27	33
8	HLDOU93	97897 02/26/97 209043 05/15/97	pCMVSPORT 3.0	18	469	1	469	77	77	327	1	44	45	88
9	HEIAX07	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	19	550	1	550	129	129	328	1			23
9	HEIAX07	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	201	376	9	376		1	510	1	8	9	15

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
10	HSAXR76	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	20	773	10	773	176	176	329	1			27
10	HSAXR76	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	202	741	55	741	190	190	511	1			27
11	HNGJJ68	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	21	991	1	991	62	62	330	1	30	31	63
11	HNGJJ68	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	203	1192	253	1137		409	512	1			19
12	HCFW04	97897 02/26/97 209043 05/15/97	pSport1	22	653	1	653	64	64	331	1	30	31	196
12	HCFW04	97897 02/26/97 209043 05/15/97	pSport1	204	589	1	513	109	109	513	1			29
13	ILMAV65	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	23	1486	596	1418	102	102	332	1	54	55	251

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
13	HLMAY65	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	205	847	1	839	87	87	514	1	30	31	74
13	HLMAY65	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	206	852	75	850		690	515	1			10
13	HTXEF04	209235 09/04/97	Uni-ZAP XR	207	1354	54	1354	100	100	516	1	33	34	206
14	HPMFD84	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	24	2323	1017	2059	1242	1242	333	1	21	22	67
14	HPMFD84	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	208	1378	113	1226	303	303	517	1	25	26	36
15	HE6DB26	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	25	683	1	683	304	304	334	1	30	31	8
15	HE6DB26	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	209	1166	281	884	567	567	518	1	18	19	19
16	HHFFL33	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	26	2036	14	1959	214	214	335	1	20	21	36

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
17	HODBD33	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	27	717	1	717	70	70	336	1	30	31	62
17	HODBD33	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	210	697	2	697	33	33	519	1	31	32	32
18	HMDAE90	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	28	495	1	495	39	39	337	1	24	25	35
19	HOUAW01	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	29	556	1	556	116	116	338	1	19	20	23
20	HBJAE44	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	30	434	1	434	78	78	339	1	35	36	40
21	HCFME41	97897 02/26/97 209043 05/15/97	pSport1	31	715	1	715	87	87	340	1	30	31	110
21	HCFME41	97897 02/26/97 209043 05/15/97	pSport1	211	932	274	932	387	387	520	1	27	28	28

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
22	HOGCO71	97897 02/26/97 209043 05/15/97	pCMVSPORT 2.0	32	486	1	486	137	137	341	1	21	22	106
23	HOSEX08	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	33	725	1	725	436	436	342	1	30	31	49
23	HOSEX08	97897 02/26/97 209043 05/15/97	Uni-ZAP XR	212	661	1	647	81	81	521	1	25	26	26
24	HSKNJ72	97897 02/26/97 209043 05/15/97	pBluescript	34	437	1	437	85	85	343	1	30	31	48
25	HEBEB69	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	35	943	1	943	196	196	344	1	30	31	40
25	HEBEB69	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	213	592	1	534	72	72	522	1	24	25	33
26	HEGEH18	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	36	604	1	604	375	375	345	1	20	21	76

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
26	HE6EH18	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	214	938	1	509		17	523	1	30	31	47
27	HSAUZ47	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	37	349	1	349	101	101	346	1	27	28	83
28	HSSDM73	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	38	672	1	672	22	22	347	1	38	39	42
29	HBMVK68	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	39	1908	135	1908	309	309	348	1	20	21	26
30	HMKDC66	97898 02/26/97 209044 05/15/97	pSport1	40	458	93	458	147	147	349	1	24	25	26
31	HMKCU94	97898 02/26/97 209044 05/15/97	pSport1	41	1153	500	1153	427	427	350	1	30	31	156
31	HMKCU94	97898 02/26/97 209044 05/15/97	pSport1	215	1079	502	896		739	524	1	23	24	43

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
32	HRDEW41	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	42	1983	1092	1983	27	27	351	1	11	12	519
32	HRDEW41	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	216	3791	2757	3357		2030	525	1			3
33	HTOJN06	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	43	1406	1	695		19	352	1	19	20	39
34	HBGDA21	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	44	1391	851	1153	74	74	353	1	30	31	233
34	HIBGDA21	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	217	1334	822	1036		638	526	1	18	19	174
35	HFGAK75	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	45	1569	768	1569	14	14	354	1	19	20	168
35	HFGAK75	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	218	1511	770	1404	844	844	527	1	32	33	43

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
36	HHPBD40	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	46	1924	1	1681	62	62	355	1	19	20	43
37	HOVCL83	97898 02/26/97 209044 05/15/97	pSport1	47	475	252	396	141	141	356	1	37	38	78
38	HBCAY62	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	48	346	1	346	61	61	357	1	19	20	24
39	HBICM48	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	49	1366	882	1300	177	177	358	1	30	31	273
39	HBICM48	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	219	642	192	581		448	528	1			13
40	HLTCL35	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	50	1405	110	1404	61	61	359	1	30	31	46
40	HLTCL35	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	220	1241	1	1241	172	172	529	1	21	22	30

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
41	HLHCK50	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	51	2633	29	310	2064	2064	360	1	18	19	117
41	HLHCK50	97898 02/26/97 209044 05/15/97	Uni-ZAP XR	221	504	207	485	222	222	530	1			3
42	HRSAN45	97899 02/26/97 209045 05/15/97	ZAP Express	52	777	1	214	113	113	361	1	24	25	52
43	HSNBB14	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	53	602	1	419	41	41	362	1	59	60	131
43	HSNBB14	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	222	1080	186	686	399	399	531	1	26	27	47
44	HMABL38	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	54	1749	222	1749	166	166	363	1	30	31	203
44	HMABL38	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	223	1258	149	1190	254	254	532	1	18	19	26

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
45	HSKDK47	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	55	1896	596	1614	650	650	364	1	33	34	47
46	HOSFH03	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	56	1753	555	1753	414	414	365	1	18	19	72
46	HOSFH03	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	224	1693	554	1693		526	533	1	25	26	58
47	HOGAV75	97899 02/26/97 209045 05/15/97	pCMVSPORT 2.0	57	1220	690	1024	128	128	366	1	30	31	101
47	HOGAV75	97899 02/26/97 209045 05/15/97	pCMVSPORT 2.0	225	1196	712	1163		1097	534	1			19
48	HFCAT74	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	58	1049	362	1049	335	335	367	1	33	34	48
49	HAGBI17	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	59	1776	854	1737	189	189	368	1	30	31	178

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
49	HAGBI17	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	226	1791	979	1791	1164	1164	535	1	18	19	40
50	HIFBC91	97899 02/26/97 209045 05/15/97	pBluescript SK-	60	443	1	443	164	164	369	1	21	22	25
51	HPRCA31	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	61	2888	1909	2888	90	90	370	1	30	31	223
51	HPRCA31	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	227	2517	1597	2517	1953	1953	536	1	18	19	57
52	HPRCE95	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	62	1851	1568	1736	139	139	371	1	30	31	348
52	HPRCE95	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	228	2424	299	2309		530	537	1	17	18	21
53	HHITLC66	97899 02/26/97 209045 05/15/97	ZAP Express	63	3542	883	3492	964	964	372	1	25	26	467

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
57	HMEFX42	97899 02/26/97 209045 05/15/97	Lambda ZAP II	233	540	25	536	227	227	542	1			20
58	IIIFHN61	97899 02/26/97 209045 05/15/97	Uni-ZAP XR	68	1751	375	1751	95	95	377	1	19	20	227
59	IICWEF90	97899 02/26/97 209045 05/15/97	ZAP Express	69	508	1	508	22	22	378	1	30	31	78
59	HCWEF90	97899 02/26/97 209045 05/15/97	ZAP Express	234	448	9	448		1	543	1	22	23	75
60	HHGCM20	97899 02/26/97 209045 05/15/97	Lambda ZAP II	70	245	1	245	93	93	379	1	28	29	50
61	HFRAU10	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	71	361	1	361	1	1	380	1	30	31	60
61	HFRAU10	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	235	407	1	407	210	210	544	1	17	18	60

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
62	HATDT67	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	72	713	8	713	169	169	381	1	30	31	39
62	HATDT67	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	236	830	190	580	329	329	545	1	28	29	39
63	HOUBG93	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	73	862	1	862	67	67	382	1	30	31	43
63	HOUBG93	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	237	932	138	905	287	287	546	1			2
64	HMWEX24	97900 02/26/97 209046 05/15/97	Uni-Zap XR	74	4602	4162	4525	730	730	383	1	30	31	202
64	HMWEX24	97900 02/26/97 209046 05/15/97	Uni-Zap XR	238	2786	2406	2739	2577	2577	547	1	22	23	36
65	HSGBA84	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	75	1255	1	1195	112	112	384	1	28	29	29

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
66	HTOCD52	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	76	475	1	475	13	13	385	1	30	31	135
66	HTOCD52	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	239	458	1	458	26	26	548	1			14
67	HTGCP16	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	77	465	25	299	74	74	386	1	33	34	41
68	HKIXR69	97900 02/26/97 209046 05/15/97	pBluescript	78	1907	1627	1730	26	26	387	1	30	31	467
68	HKIXR69	97900 02/26/97 209046 05/15/97	pBluescript	240	591	1	444	251	251	549	1			18
69	HETGJ09	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	79	1168	136	1168	267	267	388	1	20	21	29
70	HOBNC61	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	80	1285	132	1285	292	292	389	1	27	28	29

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
71	HFFAH94	97900 02/26/97 209046 05/15/97	Lambda ZAP II	81	1290	768	1054	701	701	390	1	21	22	138
72	HBIAB195	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	82	684	1	684	119	119	391	1	30	31	74
73	HSQEL25	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	83	2024	1609	1953	200	200	392	1	30	31	520
73	HSQEL25	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	241	2449	1511	2378	488	488	550	1	30	31	269
73	HSQEL25	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	242	1286	391	959		1204	551	1	9	10	10
74	HEBEG68	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	84	931	14	537	85	85	393	1	25	26	137
75	HBIAB39	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	85	825	59	802	66	66	394	1	30	31	185

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
75	HBIAB39	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	243	734	1	734	1	1	552	1	37	38	107
75	HBIAB39	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	244	809	80	794		294	553	1	15	16	106
76	HTXDU73	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	86	1238	36	918	17	17	395	1			1
77	HOEAS24	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	87	1460	9	1458	166	166	396	1	53	54	298
77	HOEAS24	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	245	2201	841	2080	507	507	554	1	43	44	135
77	HOEAS24	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	246	1661	311	1520	390	390	555	1	35	36	424
78	HTEIY30	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	88	1395	567	1395	639	639	397	1	36	37	49

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last Sig Pep	First AA of Secreted Portion	Last AA of ORF
79	HSKNE46	97900 02/26/97 209046 05/15/97	pBluescript	89	1186	352	1186	540	540	398	1	49	50	60
79	HSKNE46	97900 02/26/97 209046 05/15/97	pBluescript	247	1146	329	1146	564	564	556	1	21	22	39
80	HPMFL27	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	90	1821	1203	1614	1503	1503	399	1	30	31	79
81	HMWWDN32	97900 02/26/97 209046 05/15/97	Uni-Zap XR	91	862	253	862	359	359	400	1	32	33	36
82	HPRAX55	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	92	696	349	696	98	98	401	1	30	31	179
82	HPRAX55	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	248	1350	265	1230	348	348	557	1	32	33	58
83	HHFFW36	97900 02/26/97 209046 05/15/97	Uni-ZAP XR	93	1886	1	1759	197	197	402	1			21

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
84	HE2PL77	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	94	1774	742	1772	785	785	403	1	21	22	60
85	HSDFV29		Uni-ZAP XR	95	1779	12	1662	219	219	404	1	36	37	185
85	HSDFV29	209076 05/22/97	Uni-ZAP XR	249	2503	1	1648	206	206	558	1	32	33	151
85	HCQAV53	97901 02/26/97 209047 05/15/97	Lambda ZAP II	250	1529	72	911	191	191	559	1	20	21	33
86	HTPEG42	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	96	2801	418	2801	234	234	405	1	30	31	479
86	HTPEG42	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	251	1537	1	1537	125	125	560	1	21	22	367
87	HLHDR57	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	97	1631	916	1631	691	691	406	1	38	39	193
88	HAUAV32	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	98	504	26	504	197	197	407	1	23	24	77

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
88	HAUAV32	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	252	506	1	499	183	183	561	1	32	33	77
89	HNEBI60	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	99	1416	145	1416	456	456	408	1	18	19	73
89	HNEBI60	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	253	1348	84	1348	363	363	562	1	21	22	47
90	HSJC16	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	100	2847	1	2847		2	409	1			20
91	HTSEL31	97901 02/26/97 209047 05/15/97	pBluescript	101	1394	608	1346	602	602	410	1	23	24	87
92	HAUBL57	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	102	794	1	794	518	518	411	1	30	31	92
92	HAUBL57	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	254	1766	42	1766	356	356	563	1	30	31	167

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
92	11AUBL57	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	255	2664	47	1708		147	564	1	18	19	124
93	HODAS9	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	103	1544	898	1531	975	975	412	1			21
94	HE6CT48	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	104	871	106	871	248	248	413	1	34	35	173
94	HE6CT48	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	256	865	97	865	258	258	565	1	19	20	177
95	HMDAA61	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	105	404	1	404	16	16	414	1	21	22	63
95	HMDAA61	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	257	2082	852	2074	829	829	566	1	22	23	72
96	HAQBK61	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	106	1542	506	1542	122	122	415	1	51	52	279

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
96	HAQBK61	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	258	1482	508	1482		633	567	1	15	16	45
96	HCUHB01	209215 08/21/97	ZAP Express	259	834	1	834	82	82	568	1	40	41	251
97	HAQBF73	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	107	2327	1528	2327	465	465	416	1	30	31	283
97	HAQBF73	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	260	1508	885	1508		988	569	1			19
98	HAQBT94	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	108	1062	157	1062	172	172	417	1	28	29	187
99	HETHE07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	109	2539	275	2501	903	903	418	1	30	31	236
99	HETHE07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	261	2514	592	2431	176	176	570	1	30	31	216
99	HETHE07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	262	2357	465	2288		1151	571	1	12	13	82

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
100	HLQAB52	97901 02/26/97 209047 05/15/97	Lambda ZAP II	110	1751	969	1751	4	4	419	1	46	47	191
100	HLQAB52	97901 02/26/97 209047 05/15/97	Lambda ZAP II	263	689	218	655	314	314	572	1	18	19	95
100	HEONN58	209119 06/05/97	pSport1	264	2377	5	2377	25	25	573	1	28	29	53
101	HCRAM28	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	111	1117	1	1117		1	420	1	19	20	21
101	HIBEK16	209627 02/12/98	Other	265	1193	69	1135	242	242	574	1	24	25	107
102	HE2BG03	97901 02/26/97 209047 05/15/97	Uni-ZAP XR*	112	1313	128	1313	271	271	421	1	30	31	50
102	HE2BG03	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	266	1262	26	1262	35	35	575	1	35	36	50
103	HEBDJ82	97901 02/26/97 209047 05/15/97	Um-ZAP XR	113	1654	553	1654	709	709	422	1			32

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
104	HCUBC79	97901 02/26/97 209047 05/15/97	ZAP Express	114	1171	540	1171	337	337	423	1	30	31	162
104	HCUBC79	97901 02/26/97 209047 05/15/97	ZAP Express	267	1179	626	1161	335	335	576	1	30	31	252
104	HCUBC79	97901 02/26/97 209047 05/15/97	ZAP Express	268	1162	629	1131	942	942	577	1			18
105	HSVAF07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	115	842	373	800	100	100	424	1	65	66	173
105	HSVAF07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	269	735	290	735	25	25	578	1	65	66	80
105	HSVAF07	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	270	783	416	783		413	579	1	33	34	73
106	HT3AM65	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	116	1640	187	1470	581	581	425	1	30	31	49

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
106	HT3AM65	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	271	1638	301	1405	119	119	580	1	30	31	262
106	HT3AM65	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	272	1455	148	1188	438	438	581	1	24	25	70
107	HE6DK18	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	117	952	418	906	499	499	426	1	28	29	120
108	HEBEK93	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	118	1256	21	1079	301	301	427	1	30	31	158
108	HEBEK93	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	273	1086	25	1050	227	227	582	1	23	24	34
109	HJPCM10	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	119	1143	171	1051	175	175	428	1	50	51	153
109	HJPCM10	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	274	1003	21	1003	115	115	583	1	34	35	103

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO X	Total NT Seq	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
109	HJPCM10	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	275	1234	174	1015	232	232	584	1	27	28	132
110	HSXBL78	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	120	1782	1	1720	138	138	429	1	32	33	204
111	HOEAW81	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	121	610	18	609	50	50	430	1	30	31	66
111	HOEAW81	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	276	574	1	566	337	337	585	1	27	28	32
112	HOEAP41	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	122	526	185	375	143	143	431	1	21	22	25
113	HEAAR60	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	123	2081	1179	1976	48	48	432	1	30	31	298
113	HEAAR60	97901 02/26/97 209047 05/15/97	Uni-ZAP XR	277	1731	889	1626	886	886	586	1	18	19	28

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
114	HTXGS75	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	124	1717	764	1640	898	898	433	1	28	29	86
115	HOVBA03	97902 02/26/97 209048 05/15/97	pSport1	125	804	1	804	145	145	434	1	15	16	197
115	HOVBA03	97902 02/26/97 209048 05/15/97	pSport1	278	1320	77	637	280	280	587	1	22	23	40
116	HGBGK76	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	126	431	1	431	73	73	435	1	38	39	46
116	HGBGK76	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	279	515	1	515	43	43	588	1	20	21	30
117	HBMUW78	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	127	3752	3465	3752	748	748	436	1	30	31	369
117	IIBMUW78	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	280	2995	2738	2995	2777	2777	589	1	18	19	29

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
118	HASAS24	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	128	1144	669	1144	896	896	437	1			30
119	HSIDN55	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	129	1830	1234	1830	1265	1265	438	1			24
120	HGBGZ64	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	130	1864	1505	1741	1578	1578	439	1	37	38	53
121	H6EBJ64	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	131	2041	1	1214	46	46	440	1	35	36	175
121	H6EBJ64	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	281	1990	8	1128	71	71	590	1	16	17	92
122	HOECP43	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	132	2012	853	1986	1127	1127	441	1	22	23	77
123	H2CBV31	97902 02/26/97 209048 05/15/97	pBluescript SK-	133	1669	670	1632	962	962	442	1	25	26	32

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
124	HPCAD23	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	134	1565	281	1565	274	274	443	1	25	26	30
125	HSPAG15	97902 02/26/97 209048 05/15/97	pSport1	135	2007	1101	2007	1124	1124	444	1	39	40	69
126	HELGH31	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	136	1291	1	1180	911	911	445	1	58	59	63
127	HUSHH48	97902 02/26/97 209048 05/15/97	Lambda ZAP II	137	1906	1	1906	184	184	446	1	30	31	42
127	HUSHH48	97902 02/26/97 209048 05/15/97	Lambda ZAP II	282	2436	572	2436	726	726	591	1	30	31	42
128	HLXAU95	97902 02/26/97 209048 05/15/97	pSport1	138	1935	1044	1794	1183	1183	447	1	18	19	33
129	HHSCV65	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	139	1446	572	1347	585	585	448	1	25	26	53

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
130	HTTAD57	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	140	1109	639	1109	676	676	449	1	24	25	64
131	HEBGA37	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	141	497	9	497	95	95	450	1			34
132	HEBFU93	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	142	269	1	269	1	1	451	1	30	31	89
132	HEBFU93	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	283	782	408	781		571	592	1	31	32	70
133	HSGSC60	97902 02/26/97 209048 05/15/97	Lambda ZAP II	143	1269	55	1262	55	55	452	1	25	26	350
134	HPMGD24	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	144	1944	97	1871	306	306	453	1	16	17	49
135	HPTVC60	97902 02/26/97 209048 05/15/97	pBluescript	145	1021	526	1021	74	74	454	1	30	31	277

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
135	HPTVC60	97902 02/26/97 209048 05/15/97	pBluescript	284	961	524	961	545	545	593	1	23	24	110
136	HSKNE18	97902 02/26/97 209048 05/15/97	pBluescript	146	1285	5	1285	116	116	455	1	30	31	198
136	HSKNE18	97902 02/26/97 209048 05/15/97	pBluescript	285	1228	9	1228	324	324	594	1	26	27	30
137	HMWIF35	97902 02/26/97 209048 05/15/97	Uni-Zap XR	147	1386	169	1272	165	165	456	1	30	31	257
137	HMWIF35	97902 02/26/97 209048 05/15/97	Uni-Zap XR	286	1327	169	1208	160	160	595	1	23	24	71
138	HMWGI25	97902 02/26/97 209048 05/15/97	Uni-Zap XR	148	2098	721	2044	784	784	457	1	18	19	87
139	HSKGF03	97902 02/26/97 209048 05/15/97	pBluescript	149	1847	1689	1847	241	241	458	1	33	34	314

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
139	HSKGF03	97902 02/26/97 209048 05/15/97	pBluescript	287	1847	1033	1847	243	243	596	1	30	31	123
139	HSKGF03	97902 02/26/97 209048 05/15/97	pBluescript	288	799	1	799	243	243	597	1	12	13	47
140	HMSKE75	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	150	1569	113	1517	417	417	459	1	21	22	52
141	HCMISH30	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	151	1540	538	1540	48	48	460	1	30	31	382
141	HCMISH30	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	289	2196	270	2196	294	294	598	1	32	33	39
142	HTWCB92	97902 02/26/97 209048 05/15/97	pSport1	152	1719	690	1575	6	6	461	1	52	53	186
143	HBMDM46	97902 02/26/97 209048 05/15/97	pBluescript	153	863	1	863	195	195	462	1	26	27	162

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
143	HBMDM46	97902 02/26/97 209048 05/15/97	pBluescript	290	1185	277	1166	621	621	599	1			19
144	HFAMG13	97902 02/26/97 209048 05/15/97	Uni-ZAP XR	154	1101	1	512	40	40	463	1	21	22	46
145	HFXHL79	97903 02/26/97 209049 05/15/97	Lambda ZAP II	155	2031	669	2031	411	411	464	1	23	24	104
145	HFXHL79	97903 02/26/97 209049 05/15/97	Lambda ZAP II	291	1634	615	1485	878	878	600	1	20	21	23
146	HSNAK17	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	156	1981	1458	1809	1592	1592	465	1	23	24	69
146	HSNAK17	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	292	1795	1458	1749	1562	1562	601	1	33	34	69
147	HCFBC03	97903 02/26/97 209049 05/15/97	pSport1	157	915	45	912	22	22	466	1	22	23	154

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
147	HCFCB03	97903 02/26/97 209049 05/15/97	pSport1	293	858	46	858	224	224	602	1	30	31	77
147	HSIAP03	209139 07/03/97	Uni-ZAP XR	294	915	1	915	22	22	603	1	22	23	154
148	HSKGO26	97903 02/26/97 209049 05/15/97	pBluescript	158	2117	51	1422	32	32	467	1	23	24	332
149	HCQAV96	97903 02/26/97 209049 05/15/97	Lambda ZAP II	159	2395	14	887	722	722	468	1	22	23	48
150	HSICC16	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	160	2120	1223	2108	317	317	469	1	51	52	548
151	HTLEF62	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	161	900	482	900	46	46	470	1	30	31	285
151	HTLEF62	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	295	1517	783	1517	1062	1062	604	1			24
152	HTLAD94	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	162	1003	1	1003	288	288	471	1	30	31	79

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
152	HTLAD94	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	296	3865	217	1195	281	281	605	1	16	17	38
153	HTSFQ12	97903 02/26/97 209049 05/15/97	pBluescript	163	2196	1607	2180	1611	1611	472	1	30	31	47
154	HE6FL83	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	164	1945	271	1840	299	299	473	1	63	64	95
154	HE6FL83	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	297	1910	279	1818	355	355	606	1	39	40	69
155	HTXF155	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	165	2933	489	2871	258	258	474	1	30	31	398
155	HTXF155	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	298	3276	486	2838		525	607	1	45	46	308
156	HJPCJ76	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	166	2243	343	2221	1311	1311	475	1	20	21	45

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
157	HLTED27	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	167	1816	1130	1816	284	284	476	1	31	32	272
157	HLTED27	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	299	1695	1098	1548	1306	1306	608	1			22
158	HMKBA64	97903 02/26/97 209049 05/15/97	pSport1	168	945	1	787	208	208	477	1	18	19	192
159	HNFIP24	97903 02/26/97 209049 05/15/97	pBluescript	169	902	46	816	19	19	478	1	26	27	234
160	HCELB21	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	170	1883	798	1869	1001	1001	479	1	45	46	104
160	HCELB21	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	300	1501	438	1501	510	510	609	1			24
161	HAWBA28	97903 02/26/97 209049 05/15/97	pBluescript SK-	171	2100	1642	2100	1722	1722	480	1	23	24	32

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO-X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
162	HSAAS44	97903 02/26/97 209049 05/15/97	pBluescript SK-	172	1930	187	1930	65	65	481	1	30	31	570
162	HSAAS44	97903 02/26/97 209049 05/15/97	pBluescript SK-	301	2683	183	2683	431	431	610	1			24
163	HAFAL73	97903 02/26/97 209049 05/15/97	pBluescript SK-	173	1509	962	1451	122	122	482	1	30	31	311
163	HAFAL73	97903 02/26/97 209049 05/15/97	pBluescript SK-	302	1454	961	1420	976	976	611	1			1
164	HSAWF26	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	174	3173	2197	2972	51	51	483	1	21	22	328
164	HSAWF26	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	303	828	52	828	305	305	612	1			8
165	IIEAAL31	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	175	991	374	970	60	60	484	1	24	25	177

Gene No	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
165	HEAAL31	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	304	2416	1387	2413	1473	1473	613	1	18	19	25
166	HFKFX55	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	176	1290	499	1290	130	130	485	1	19	20	238
167	H2LAO11	97903 02/26/97 209049 05/15/97	pBluescript SK-	177	2290	1	2290	173	173	486	1	22	23	62
168	HPFDZ95	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	178	549	1	549	11	11	487	1	21	22	27
168	HPFDZ95	97903 02/26/97 209049 05/15/97	Uni-ZAP XR	305	545	1	545	17	17	614	1	21	22	27
169	HPTTU11	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	179	1509	294	1352	92	92	488	1	30	31	338
169	HPTTU11	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	306	1530	385	1530	562	562	615	1	23	24	61

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq	5' NT of Clone Seq.	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
170	HCFAE79	97904 02/26/97 209050 05/15/97	pSport1	180	1316	985	1250	995	995	489	1	26	27	32
171	HTEDJ34	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	181	777	1	777	51	51	490	1	30	31	47
171	HTEDJ34	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	307	997	244	997	300	300	616	1	23	24	29
172	HODCW06	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	182	791	1	791	14	14	491	1	29	30	38
173	HFTAR26	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	183	1405	346	1405	575	575	492	1	20	21	61
174	H2MBF44	97904 02/26/97 209050 05/15/97	pBluescript SK-	184	1596	75	1596	131	131	493	1	24	25	345
174	H2MBF44	97904 02/26/97 209050 05/15/97	pBluescript SK-	308	2345	75	2345	233	233	617	1	56	57	69

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
175	HE8BI92	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	185	2293	355	2288	67	67	494	1	30	31	236
175	HE8BI92	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	309	2369	2	1946		60	618	1	9	10	24
176	HFTBR48	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	186	1212	462	1180	257	257	495	1	30	31	199
176	HFTBR48	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	310	1181	424	1149	663	663	619	1	23	24	35
177	HE9CM64	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	187	1605	770	1554	166	166	496	1	30	31	350
177	HE9CM64	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	311	1537	719	1515		787	620	1	43	44	130
178	HAATAV51	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	188	1516	960	1516	8	8	497	1	30	31	264

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO.X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO.Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
178	HATAV51	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	312	1493	1	1261	54	54	621	1	18	19	23
179	HAQAF27	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	189	681	287	681		401	498	1			25
180	HCEEK08	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	190	1014	703	1014	360	360	499	1	30	31	158
180	HCEEK08	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	313	577	1	577		175	622	1			6
181	HAFU18	97904 02/26/97 209050 05/15/97	pBluescript SK-	191	2779	2207	2630	1153	1153	500	1	30	31	278
181	HAFU18	97904 02/26/97 209050 05/15/97	pBluescript SK-	314	2860	163	2860	21	21	623	1	30	31	231
181	HAFU18	97904 02/26/97 209050 05/15/97	pBluescript SK-	315	876	275	876	302	302	624	1	32	33	34

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
182	HETBY74	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	192	1923	30	1923	45	45	501	1	33	34	193
183	HTOAF35	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	193	2346	1160	2286	178	178	502	1	30	31	204
183	HTOAF35	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	316	2025	840	2025	971	971	625	1	18	19	21
184	HCRBX32	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	194	3054	2004	3054	434	434	503	1	11	12	146
184	HCRBX32	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	317	3026	1966	3026		2131	626	1			9
185	HEBGB80	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	195	907	152	907	297	297	504	1	30	31	63
185	HEBGB80	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	318	712	67	712	107	107	627	1	18	19	29

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq	3' NT of Clone Seq	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
186	HFAMH74	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	196	1290	84	809	225	225	505	1	30	31	93
186	HFAMH74	97904 02/26/97 209050 05/15/97	Uni-ZAP XR	319	1289	785	1289	927	927	628	1	28	29	30

5

10

15

20

25

30

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also

5 hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

10 Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the

15 actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the

20 generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.

25 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

30 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of

predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

5 In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis
10 of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence
15 shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present
20 invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of
25 directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present
30 invention.

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

5 The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many
10 regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the
15 nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).
20 Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%,
25 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95%
30 "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each

100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then

subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference

09882171.061801
108190" T28860

sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal

residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are

09882171.061601

included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or

fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this

context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed

5 herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the
10 cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,
15 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

20 Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-
25 60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also
30 preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and

alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

5 Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily
10 identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a
15 polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a
20 polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments,
25 variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using
30 techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using

09882171 061901

colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses

polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention

encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA

81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,

that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985)). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μ g of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an

immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example, polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof, resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.* 8:724-33

(1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin

molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be

excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog,

ortholog, or homolog of a polypeptide of the present invention are included.

Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present

invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using

methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent

hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide

of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the

epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes
5 antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand
10 binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described
15 supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent
20 ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but
25 do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists
30 for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.

5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entirety).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of

numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

5 The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal
10 antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and
15 potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be
20 produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not
25 limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma
30 technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an

immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are
5 selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating
10 monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody
15 able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).
20 F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which
25 carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid
30 surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage

gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol.*

09882171 061801
"T 278860"

Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into

mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using

0982171 051801
FOB FSD " F F28860

techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library

09882171 061801

generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe
5 specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the
10 antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor,
15 NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light
20 chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted
25 within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of
30 the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino

acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038-1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a

polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as

bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems
 5 infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,
 10 BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole
 15 recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2
 20 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which
 25 direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic
 30 Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such

fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt,

which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 5 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. 10 (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

15 The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in 20 culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second 25 vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic 30 free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entirety.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any

combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341 (1992) (said references incorporated by reference in their entirety).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to

identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred
5 embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags
10 useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used
15 diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent
20 materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No.
25 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone,
30 fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin,

and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific

epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A

and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes

the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any

one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention,

including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other

desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific

5. embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or
10 indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be
15 accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun;
20 Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target
25 cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT
30 Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination

(Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages,

neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment;

this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by

use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of

the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods

including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with

a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may

also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound

recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

5 Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target
10 cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur
15 through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell
20 or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and
25 specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins
30 consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)

Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral
5 vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate,
10 such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac
15 promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a
20 translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin
25 resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as
30 CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated

or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1*

"Pichia" Protocols: Methods in Molecular Biology

promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, 5 pPHIL-D2, pPHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding 10 sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs 15 discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and 20 which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 25 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using 30 techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and

09682171.061801

Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH_4 ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as

09882171.061801

increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG

to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated

5 polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for

10 therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid,

15 glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

20 One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be

25 performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be

30 accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions,

substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional
5 embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as,
10 for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion
15 protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (*e.g.*, that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one
20 instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (*i.e.*, naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the
25 heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, *e.g.*, US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as
30 described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for

example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous

polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers
5 of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues

10 located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers
15 containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its
20 entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent
25 Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse
30 orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described

herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by
5 reference in its entirety).

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes
10 known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present
15 invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of
20 somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per
25 day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends
30 Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see
 5 Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

10 The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-
 15 492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage
 20 analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated
 25 with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or
 30 translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide

and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or

estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard
5 being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

10 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue
15 sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic
20 method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with
25 polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

30 The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the

preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ($T_{sub.m}$) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al.,

"The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by
 5 insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in
 10 some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International
 15 Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580;
 20 Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit
 25 proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca
 30 Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the

polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456
5 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into
10 polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective
15 gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute
20 biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of
25 "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative
30 to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a

unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics

of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldgrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

09882171.061801

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired

5 polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

30 The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or

bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of

DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

5 Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE),
10 among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine
15 (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2
20 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available
25 to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology, 101:512-527 (1983),
30 which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated.

SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ratio will be from about 5:1 to about 1:5. More preferably, the ratio will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors

may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

5 The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by
10 reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

15 The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with
20 polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional
25 mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science , 252:431-434 (1991);
30 Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: Ela, Elb, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor

Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses.

5 Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

10 Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO
15 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the
20 art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous
25 polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same
30 restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the

09882171-061801

amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle

accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA, 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a

polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with the following systems.

Immune Activity

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis,

producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

5 Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare
10 lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

15 In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, and/or agonists or
20 antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic
25 alymphoplasia-aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented,
30 diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, diagnosed and/or prognosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura), autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, neuritis, uveitis ophthalmia, polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary

09882171.061801

inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, prognosing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, Polynucleotides, polypeptides, antibodies, and/or

agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, may be used to treat, prevent, diagnose and/or prognose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain

injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are

5 characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

Because inflammation is a fundamental defense mechanism, inflammatory

10 disorders can effect virtually any tissue of the body. Accordingly, polynucleotides, polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adenitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis,

15 chorditis, cochitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis,

20 peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose,

25 prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues.

Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or

30 antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides,

antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

5 In other embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

10 Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an
15 existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

20 In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art.

30 In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In

09882171 061801

another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, 5 cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal 10 immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group 15 consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meissneria meningitidis*, 20 *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may 25 be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an 30 immune response to Plasmodium (malaria) or Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat

0988217.1 061801
T08T90" T2T8860

infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

10 In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an
15 immune response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell responsiveness to pathogens.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that elevates the immune status of an individual prior to their receipt of
25 immunosuppressive therapies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce higher affinity antibodies.

30 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to accelerate recovery of immunocompromised individuals.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

10 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, 15 compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists 30 or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious

mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one
5 embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to
10 modulate the immune system.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

15 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more
20 rapidly their susceptibility profile would likely change.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in the
30 pretreatment of bone marrow samples prior to transplant.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based

09882171 051871

therapy for genetically inherited disorders resulting in immuno-
incompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a means of
5 activating monocytes/macrophages to defend against parasitic diseases that effect
monocytes such as Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a means of
regulating secreted cytokines that are elicited by polypeptides of the invention.

10 In another embodiment, polypeptides, antibodies, polynucleotides and/or
agonists or antagonists of the present invention are used in one or more of the
applications described herein, as they may apply to veterinary medicine.

In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a means of
15 blocking various aspects of immune responses to foreign agents or self. Examples of
diseases or conditions in which blocking of certain aspects of immune responses may
be desired include autoimmune disorders such as lupus, and arthritis, as well as
immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and
diseases/disorders associated with pathogens.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a therapy for
preventing the B cell proliferation and Ig secretion associated with autoimmune
diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus
and multiple sclerosis.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a inhibitor of B
and/or T cell migration in endothelial cells. This activity disrupts tissue architecture
or cognate responses and is useful, for example in disrupting immune responses, and
blocking sepsis.

30 In another specific embodiment, polypeptides, antibodies, polynucleotides
and/or agonists or antagonists of the present invention are used as a therapy for
chronic hypergammaglobulinemia evident in such diseases as monoclonal

gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance
5 to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

10 The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit
15 complement mediated cell lysis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for
20 treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult
25 respiratory distress syndrome (ARDS).

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of
30 vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to diagnose, prognose, treat, and/or prevent a disorder characterized

09882171.061801
T09907228660

by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with polynucleotides or polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

10 In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

15 Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of the present invention (e.g., Fc fusion protein; see, e.g., Example 9). Agonists of the invention include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins; see, e.g., Example 9). polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

20 In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of
25 producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741). Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of
30 monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention in an organ system listed above.

Blood-Related Disorders

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hemostatic (the stopping of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes.

Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to

prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes,

lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of anemias and leukopenias described below. Alternatively, the

polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for

example eosinophilia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, treat, or diagnose blood dyscrasia.

Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious

anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hemolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias arising from drug treatments such as anemias associated with methyl dopa, dapsone, and/or sulfadiazine. Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating thalassemias, including, but not limited to major and minor forms of alpha-thalassemia and beta-thalassemia.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thrombasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophilias such as hemophilia A or Factor VII deficiency and Christmas disease or Factor IX deficiency, Hereditary

Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

The effect of the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention on the clotting time of blood may be monitored using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in a specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction accompanying kidney failure, leukemia, multiple myeloma, cirrhosis of the liver, and systemic lupus erythematosus as well as platelet dysfunction associated with drug treatments, including treatment with aspirin, ticlopidine, nonsteroidal anti-inflammatory drugs (used for arthritis, pain, and sprains), and penicillin in high doses.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders characterized by or associated with increased or decreased numbers of white blood cells. Leukopenia occurs when the number of white blood cells decreases below normal. Leukopenias include, but are not limited to, neutropenia and lymphocytopenia. An increase in the number of white blood cells compared to normal is known as leukocytosis. The body generates increased numbers of white blood cells during infection. Thus, leukocytosis may simply be a normal physiological parameter that reflects infection. Alternatively, leukocytosis may be an indicator of injury or other disease such as cancer.

Leukocytoses, include but are not limited to, eosinophilia, and accumulations of macrophages. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukopenia. In other specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or

098827.061901
T03T90 T28860

antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukocytosis.

Leukopenia may be a generalized decreased in all types of white blood cells, or may be a specific depletion of particular types of white blood cells. Thus, in specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating decreases in neutrophil numbers, known as neutropenia. Neutropenias that may be diagnosed, prognosed, prevented, and/or treated by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, infantile genetic agranulocytosis, familial neutropenia, cyclic neutropenia, neutropenias resulting from or associated with dietary deficiencies (e.g., vitamin B 12 deficiency or folic acid deficiency), neutropenias resulting from or associated with drug treatments (e.g., antibiotic regimens such as penicillin treatment, sulfonamide treatment, anticoagulant treatment, anticonvulsant drugs, anti-thyroid drugs, and cancer chemotherapy), and neutropenias resulting from increased neutrophil destruction that may occur in association with some bacterial or viral infections, allergic disorders, autoimmune diseases, conditions in which an individual has an enlarged spleen (e.g., Felty syndrome, malaria and sarcoidosis), and some drug treatment regimens.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating lymphocytopenias (decreased numbers of B and/or T lymphocytes), including, but not limited lymphocytopenias resulting from or associated with stress, drug treatments (e.g., drug treatment with corticosteroids, cancer chemotherapies, and/or radiation therapies), AIDS infection and/or other diseases such as, for example, cancer, rheumatoid arthritis, systemic lupus erythematosus, chronic infections, some viral infections and/or hereditary disorders (e.g., DiGeorge syndrome, Wiskott-Aldrich Syndrome, severe combined immunodeficiency, ataxia telangiectasia).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with macrophage numbers and/or

macrophage function including, but not limited to, Gaucher's disease, Niemann-Pick disease, Letterer-Siwe disease and Hand-Schuller-Christian disease.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing,
 5 prognosing, preventing, and/or treating diseases and disorders associated with eosinophil numbers and/or eosinophil function including, but not limited to, idiopathic hypereosinophilic syndrome, eosinophilia-myalgia syndrome, and Hand-Schuller-Christian disease.

In yet another embodiment, the polynucleotides, polypeptides, antibodies,
 10 and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukemias and lymphomas including, but not limited to, acute lymphocytic (lymphoblastic) leukemia (ALL), acute myeloid (myelocytic, myelogenous, myeloblastic, or myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., B cell leukemias, T cell leukemias, Sezary syndrome, and
 15 Hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, or granulocytic) leukemia, Hodgkin's lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, and mycosis fungoides.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing,
 20 prognosing, preventing, and/or treating diseases and disorders of plasma cells including, but not limited to, plasma cell dyscrasias, monoclonal gammaopathies, monoclonal gammopathies of undetermined significance, multiple myeloma, macroglobulinemia, Waldenstrom's macroglobulinemia, cryoglobulinemia, and Raynaud's phenomenon.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing,
 25 and/or diagnosing myeloproliferative disorders, including but not limited to, polycythemia vera, relative polycythemia, secondary polycythemia, myelofibrosis, acute myelofibrosis, agnogenic myeloid metaplasia, thrombocythemia, (including both
 30 primary and secondary thrombocythemia) and chronic myelocytic leukemia.

09882171.051801

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as a treatment prior to surgery, to increase blood cell production.

5 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to enhance the migration, phagocytosis, superoxide production, antibody dependent cellular cytotoxicity of neutrophils, eosinophils and macrophages.

10 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to stem cells pheresis. In another specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to platelet pheresis.

15 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase cytokine production.

20 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, diagnosing, and/or treating primary hematopoietic disorders.

Hyperproliferative Disorders

25 In certain embodiments, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

30 For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune

response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue

- Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive

05832171 051301
T02T50 T22886

Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal
 5 Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In another preferred embodiment, polynucleotides or polypeptides, or agonists or antagonists of the present invention are used to diagnose, prognose, prevent, and/or
 10 treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders described above. Such uses are indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal
 15 growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W. B. Saunders Co., Philadelphia, pp. 68-79.)

Hyperplasia is a form of controlled cell proliferation, involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Hyperplastic disorders which can be diagnosed, prognosed, prevented, and/or treated
 20 with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, angiofollicular mediastinal lymph node hyperplasia, angiolymphoid hyperplasia with eosinophilia, atypical melanocytic hyperplasia, basal cell hyperplasia, benign giant lymph node hyperplasia, cementum hyperplasia, congenital adrenal hyperplasia, congenital sebaceous hyperplasia, cystic
 25 hyperplasia, cystic hyperplasia of the breast, denture hyperplasia, ductal hyperplasia, endometrial hyperplasia, fibromuscular hyperplasia, focal epithelial hyperplasia, gingival hyperplasia, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, intravascular papillary endothelial hyperplasia, nodular hyperplasia of prostate, nodular regenerative hyperplasia, pseudoepitheliomatous hyperplasia,
 30 senile sebaceous hyperplasia, and verrucous hyperplasia.

Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplastic disorders

09882771.061801
 108190.72886

which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, agnogenic myeloid metaplasia, apocrine metaplasia, atypical metaplasia, autoparenchymatous metaplasia, connective tissue metaplasia, epithelial metaplasia, intestinal metaplasia, metaplastic anemia, metaplastic ossification, metaplastic polyps, myeloid metaplasia, primary myeloid metaplasia, secondary myeloid metaplasia, squamous metaplasia, squamous metaplasia of amnion, and symptomatic myeloid metaplasia.

Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation. Dysplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, anhidrotic ectodermal dysplasia, anterofacial dysplasia, asphyxiating thoracic dysplasia, atridigital dysplasia, bronchopulmonary dysplasia, cerebral dysplasia, cervical dysplasia, chondroectodermal dysplasia, cleidocranial dysplasia, congenital ectodermal dysplasia, craniodiaphysial dysplasia, craniocarpotarsal dysplasia, craniometaphysial dysplasia, dentin dysplasia, diaphysial dysplasia, ectodermal dysplasia, enamel dysplasia, encephalo-ophthalmic dysplasia, dysplasia epiphysialis hemimelia, dysplasia epiphysialis multiplex, dysplasia epiphysialis punctata, epithelial dysplasia, faciodigitogenital dysplasia, familial fibrous dysplasia of jaws, familial white folded dysplasia, fibromuscular dysplasia, fibrous dysplasia of bone, florid osseous dysplasia, hereditary renal-retinal dysplasia, hidrotic ectodermal dysplasia, hypohidrotic ectodermal dysplasia, lymphopenic thymic dysplasia, mammary dysplasia, mandibulofacial dysplasia, metaphysial dysplasia, Mondini dysplasia, monostotic fibrous dysplasia, mucoepithelial dysplasia, multiple epiphysial dysplasia, oculoauriculovertebral dysplasia, oculodentodigital dysplasia, oculovertbral dysplasia, odontogenic dysplasia, ophthalmomandibulomelic dysplasia, periapical cemental dysplasia, polyostotic fibrous dysplasia,

05632171.061B01

pseudoachondroplastic spondyloepiphyseal dysplasia, retinal dysplasia, septo-optic dysplasia, spondyloepiphyseal dysplasia, and ventriculoradial dysplasia.

Additional pre-neoplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including

5 polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, benign dysproliferative disorders (e.g., benign tumors, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, and esophageal dysplasia), leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis.

In another embodiment, a polypeptide of the invention, or polynucleotides,

10 antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

In another embodiment, polynucleotides, polypeptides, antibodies, and/or

15 agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat cancers and neoplasms, including, but not limited to those described herein. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein,

20 may be used to treat acute myelogenous leukemia.

Additionally, polynucleotides, polypeptides, and/or agonists or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the

25 inhibition of apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer,

30 intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and

09882171.061801

ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

In preferred embodiments, polynucleotides, polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma,

pinealoma, emangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or

5 agonists or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease,

10 polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-

15 induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, neoplasms

20 located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be diagnosed, prognosed,

25 prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other

30 hyperproliferative disease, besides neoplasia, located in an organ system listed above.

09882171 051801
T 08190 T 228860

pinealoma, emangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, neoplasms located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

09882171.061801

Another preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells.

10 In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably
15 an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other
20 polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the
25 present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the
30 destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By

"biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present

invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than $5 \times 10^{-6} \text{M}$, 10^{-6}M , $5 \times 10^{-7} \text{M}$, 10^{-7}M , $5 \times 10^{-8} \text{M}$, 10^{-8}M , $5 \times 10^{-9} \text{M}$, 10^{-9}M , $5 \times 10^{-10} \text{M}$, 10^{-10}M , $5 \times 10^{-11} \text{M}$, 10^{-11}M , $5 \times 10^{-12} \text{M}$, 10^{-12}M , $5 \times 10^{-13} \text{M}$, 10^{-13}M , $5 \times 10^{-14} \text{M}$, 10^{-14}M , $5 \times 10^{-15} \text{M}$, and 10^{-15}M .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, anti-inflammatory proteins

(See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

5 Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4
10 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions
15 containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic
20 and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and
25 immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Renal Disorders

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the
30 present invention, may be used to treat, prevent, diagnose, and/or prognose disorders of the renal system. Renal disorders which can be diagnosed, prognosed, prevented,

and/or treated with compositions of the invention include, but are not limited to, kidney failure, nephritis, blood vessel disorders of kidney, metabolic and congenital kidney disorders, urinary disorders of the kidney, autoimmune disorders, sclerosis and necrosis, electrolyte imbalance, and kidney cancers.

5 Kidney diseases which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, acute kidney failure, chronic kidney failure, atheroembolic renal failure, end-stage renal disease, inflammatory diseases of the kidney (e.g., acute glomerulonephritis, postinfectious glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome, 10 membranous glomerulonephritis, familial nephrotic syndrome, membranoproliferative glomerulonephritis I and II, mesangial proliferative glomerulonephritis, chronic glomerulonephritis, acute tubulointerstitial nephritis, chronic tubulointerstitial nephritis, acute post-streptococcal glomerulonephritis (PSGN), pyelonephritis, lupus nephritis, chronic nephritis, interstitial nephritis, and post-streptococcal 15 glomerulonephritis), blood vessel disorders of the kidneys (e.g., kidney infarction, atheroembolic kidney disease, cortical necrosis, malignant nephrosclerosis, renal vein thrombosis, renal underperfusion, renal retinopathy, renal ischemia-reperfusion, renal artery embolism, and renal artery stenosis), and kidney disorders resulting from urinary tract disease (e.g., pyelonephritis, hydronephrosis, urolithiasis (renal lithiasis, 20 nephrolithiasis), reflux nephropathy, urinary tract infections, urinary retention, and acute or chronic unilateral obstructive uropathy.)

 In addition, compositions of the invention can be used to diagnose, prognose, prevent, and/or treat metabolic and congenital disorders of the kidney (e.g., uremia, renal amyloidosis, renal osteodystrophy, renal tubular acidosis, renal glycosuria, 25 nephrogenic diabetes insipidus, cystinuria, Fanconi's syndrome, renal fibrocystic osteosis (renal rickets), Hartnup disease, Bartter's syndrome, Liddle's syndrome, polycystic kidney disease, medullary cystic disease, medullary sponge kidney, Alport's syndrome, nail-patella syndrome, congenital nephrotic syndrome, CRUSH syndrome, horseshoe kidney, diabetic nephropathy, nephrogenic diabetes insipidus, 30 analgesic nephropathy, kidney stones, and membranous nephropathy), and autoimmune disorders of the kidney (e.g., systemic lupus erythematosus (SLE),

Goodpasture syndrome, IgA nephropathy, and IgM mesangial proliferative glomerulonephritis).

Compositions of the invention can also be used to diagnose, prognose, prevent, and/or treat sclerotic or necrotic disorders of the kidney (e.g.,

glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), necrotizing glomerulonephritis, and renal papillary necrosis), cancers of the kidney (e.g., nephroma, hypernephroma, nephroblastoma, renal cell cancer, transitional cell cancer, renal adenocarcinoma, squamous cell cancer, and Wilm's tumor), and electrolyte imbalances (e.g., nephrocalcinosis, pyuria, edema, hydronephritis, proteinuria, hyponatremia, hypernatremia, hypokalemia, hyperkalemia, hypocalcemia, hypercalcemia, hypophosphatemia, and hyperphosphatemia).

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose cardiovascular disorders, including, but not limited to, peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include, but are not limited to, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include, but are not limited to, aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia,

patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects,

- 5 Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include, but are not limited to, heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac
10 edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease,
15 ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include, but are not limited to, sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine
20 Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia,
25 Torsades de Pointes, and ventricular tachycardia.

Heart valve diseases include, but are not limited to, aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia,
30 tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include, but are not limited to, alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular

stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include, but are not limited to, coronary disease, such as
 5 angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema,
 10 aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's
 15 disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include, but are not limited to, dissecting aneurysms, false
 20 aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include, but are not limited to, arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery
 25 occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include, but are not limited to, carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis,
 30 Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia

(including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include, but are not limited to, air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include, but are not limited to, coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemic disorders include, but are not limited to, cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes, but is not limited to, aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

Respiratory Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be used to treat, prevent, diagnose, and/or prognose diseases and/or disorders of the respiratory system.

Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis, juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's

nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomonas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal infections in people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus spp.*; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia spp.*), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis,

desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present

invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*,

5 Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists
10 may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder,
15 thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and
20 Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a
25 catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These
30 disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; atherosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy,

retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists of the invention are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

09882171-061801
T08T90-TZT880

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion,

but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or

agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated, prevented, diagnosed, and/or prognosed with the the polynucleotides, polypeptides, agonists and/or antagonists of the invention include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or antagonists may also be used in controlling menstruation or administered as either a

09882171.061801

peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch
5 granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal
10 surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic
15 compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-
20 angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the
25 site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly
30 preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum

098217061801
T08F90" T ZT8860

(VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

5 A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence
10 of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem.
15 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987);
20 Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

25 **Diseases at the Cellular Level**

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include
30 cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma,

lymphoma, endothelioma, osteblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma,

craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or

antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentoplastic graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis.

5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat
10 gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or
15 antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect
20 the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associated with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to
25 various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke
30 inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as

agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neural Activity and Neurological Diseases

The polynucleotides, polypeptides and agonists or antagonists of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system disorders that can be treated with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the methods of the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or

ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In one embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of hypoxia. In a further preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated

with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, 10:3507-15 (1990); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, 70:65-82 (1980), or Brown *et al.*, *Ann. Rev.*

09882171.061801

Neurosci., 4:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Further, polypeptides or polynucleotides of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including polynucleotides, polypeptides, and agonists or antagonists) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, polypeptides, polynucleotides and/or agonists or antagonists of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell

leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention

0982171 061301
T 228860

5

10

25

30

transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon-Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolysaccharidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydranencephaly, Arnold-Chiari Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman

syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia,
 myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such
 as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which
 includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as
 5 diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external
 ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic
 Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory
 paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as
 ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision
 10 defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as
 hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism,
 spasm such as trismus, unconsciousness such as coma, persistent vegetative state and
 syncope and vertigo, neuromuscular diseases such as amyotonia congenita,
 amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron
 15 disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease
 and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy,
 Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy,
 Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis
 and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia,
 20 amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome,
 Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex
 Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as
 Acoustic Nerve Diseases such as Acoustic Neuroma which includes
 Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-
 25 Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus,
 oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's
 Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns
 Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis,
 Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic
 30 Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica,
 Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases

such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Endocrine Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose disorders and/or diseases related to hormone imbalance, and/or disorders or diseases of the endocrine system.

Hormones secreted by the glands of the endocrine system control physical growth, sexual function, metabolism, and other functions. Disorders may be classified in two ways: disturbances in the production of hormones, and the inability of tissues to respond to hormones. The etiology of these hormone imbalance or endocrine system diseases, disorders or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy, injury or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular disease or disorder related to the endocrine system and/or hormone imbalance.

Endocrine system and/or hormone imbalance and/or diseases encompass disorders of uterine motility including, but not limited to: complications with

09632171.061804
1008190.1.061804

pregnancy and labor (e.g., pre-term labor, post-term pregnancy, spontaneous abortion, and slow or stopped labor); and disorders and/or diseases of the menstrual cycle (e.g., dysmenorrhea and endometriosis).

Endocrine system and/or hormone imbalance disorders and/or diseases include

5 disorders and/or diseases of the pancreas, such as, for example, diabetes mellitus, diabetes insipidus, congenital pancreatic agenesis, pheochromocytoma--islet cell tumor syndrome; disorders and/or diseases of the adrenal glands such as, for example, Addison's Disease, corticosteroid deficiency, virilizing disease, hirsutism, Cushing's Syndrome, hyperaldosteronism, pheochromocytoma; disorders and/or diseases of the

10 pituitary gland, such as, for example, hyperpituitarism, hypopituitarism, pituitary dwarfism, pituitary adenoma, panhypopituitarism, acromegaly, gigantism; disorders and/or diseases of the thyroid, including but not limited to, hyperthyroidism, hypothyroidism, Plummer's disease, Graves' disease (toxic diffuse goiter), toxic nodular goiter, thyroiditis (Hashimoto's thyroiditis, subacute granulomatous

15 thyroiditis, and silent lymphocytic thyroiditis), Pendred's syndrome, myxedema, cretinism, thyrotoxicosis, thyroid hormone coupling defect, thymic aplasia, Hurthle cell tumours of the thyroid, thyroid cancer, thyroid carcinoma, Medullary thyroid carcinoma; disorders and/or diseases of the parathyroid, such as, for example, hyperparathyroidism, hypoparathyroidism; disorders and/or diseases of the

20 hypothalamus.

In specific embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists of those polypeptides (including antibodies) as well as fragments and variants of those polynucleotides, polypeptides, agonists and antagonists, may be used to diagnose, prognose, treat,

25 prevent, or ameliorate diseases and disorders associated with aberrant glucose metabolism or glucose uptake into cells.

In a specific embodiment, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists and/or antagonists thereof may be used to diagnose, prognose, treat, prevent, and/or ameliorate type I diabetes mellitus (insulin

30 dependent diabetes mellitus, IDDM).

In another embodiment, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists and/or antagonists thereof may be used to

diagnose, prognose, treat, prevent, and/or ameliorate type II diabetes mellitus (insulin resistant diabetes mellitus).

Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or antagonists thereof (especially neutralizing or antagonistic antibodies) may be used to diagnose, prognose, treat, prevent, and/or ameliorate conditions associated with (type I or type II) diabetes mellitus, including, but not limited to, diabetic ketoacidosis, diabetic coma, nonketotic hyperglycemic-hyperosmolar coma, seizures, mental confusion, drowsiness, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section), dyslipidemia, kidney disease (e.g., renal failure, nephropathy other diseases and disorders as described in the "Renal Disorders" section), nerve damage, neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture.

In other embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to regulate the animal's weight. In specific embodiments the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to control the animal's weight by modulating a biochemical pathway involving insulin. In still other embodiments the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to control the animal's weight by modulating a biochemical pathway involving insulin-like growth factor.

In addition, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases of the testes or ovaries, including cancer. Other disorders and/or diseases of the testes or ovaries further include, for example, ovarian cancer, polycystic ovary syndrome, Klinefelter's syndrome, vanishing testes syndrome (bilateral anorchia), congenital absence of Leydig's cells,

cryptorchidism, Noonan's syndrome, myotonic dystrophy, capillary haemangioma of the testis (benign), neoplasias of the testis and neo-testis.

Moreover, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases such as, for example,

5 polyglandular deficiency syndromes, pheochromocytoma, neuroblastoma, multiple Endocrine neoplasia, and disorders and/or cancers of endocrine tissues.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose, prognose, prevent, and/or treat endocrine diseases and/or disorders

10 associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

Reproductive System Disorders

15 The polynucleotides or polypeptides, or agonists or antagonists of the invention may be used for the diagnosis, treatment, or prevention of diseases and/or disorders of the reproductive system. Reproductive system disorders that can be treated by the compositions of the invention, include, but are not limited to, reproductive system injuries, infections, neoplastic disorders, congenital defects, and

20 diseases or disorders which result in infertility, complications with pregnancy, labor, or parturition, and postpartum difficulties.

Reproductive system disorders and/or diseases include diseases and/or disorders of the testes, including testicular atrophy, testicular feminization, cryptorchism (unilateral and bilateral), anorchia, ectopic testis, epididymitis and

25 orchitis (typically resulting from infections such as, for example, gonorrhea, mumps, tuberculosis, and syphilis), testicular torsion, vasitis nodosa, germ cell tumors (e.g., seminomas, embryonal cell carcinomas, teratocarcinomas, choriocarcinomas, yolk sac tumors, and teratomas), stromal tumors (e.g., Leydig cell tumors), hydrocele, hematocele, varicocele, spermatocele, inguinal hernia, and disorders of sperm

30 production (e.g., immotile cilia syndrome, aspermia, asthenozoospermia, azoospermia, oligospermia, and teratozoospermia).

5

10

25

30

present invention may be used in the diagnosis, treatment, and/or prevention of

diseases and/or disorders of the vagina and vulva, including bacterial vaginosis, candida vaginitis, herpes simplex virus, chancroid, granuloma inguinale, lymphogranuloma venereum, scabies, human papillomavirus, vaginal trauma, vulvar trauma, adenositis, chlamydia vaginitis, gonorrhea, trichomonas vaginitis, condyloma acuminatum, syphilis, molluscum contagiosum, atrophic vaginitis, Paget's disease, lichen sclerosus, lichen planus, vulvodynia, toxic shock syndrome, vaginismus, vulvovaginitis, vulvar vestibulitis, and neoplastic disorders, such as squamous cell hyperplasia, clear cell carcinoma, basal cell carcinoma, melanomas, cancer of Bartholin's gland, and vulvar intraepithelial neoplasia.

Disorders and/or diseases of the uterus include dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding (e.g., due to aberrant hormonal signals), and neoplastic disorders, such as adenocarcinomas, leiomyosarcomas, and sarcomas. Additionally, the polypeptides, polynucleotides, or agonists or antagonists of the invention may be useful as a marker or detector of, as well as in the diagnosis, treatment, and/or prevention of congenital uterine abnormalities, such as bicornuate uterus, septate uterus, simple unicornuate uterus, unicornuate uterus with a noncavitary rudimentary horn, unicornuate uterus with a non-communicating cavitary rudimentary horn, unicornuate uterus with a communicating cavitary horn, arcuate uterus, uterine didelphys, and T-shaped uterus.

Ovarian diseases and/or disorders include anovulation, polycystic ovary syndrome (Stein-Leventhal syndrome), ovarian cysts, ovarian hypofunction, ovarian insensitivity to gonadotropins, ovarian overproduction of androgens, right ovarian vein syndrome, amenorrhea, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, Sertoli-Leydig tumors, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, and Ovarian Krukenberg tumors).

Cervical diseases and/or disorders include cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, and cervical neoplasms (including, for example,

cervical carcinoma, squamous metaplasia, squamous cell carcinoma, adenosquamous cell neoplasia, and columnar cell neoplasia).

Additionally, diseases and/or disorders of the reproductive system include disorders and/or diseases of pregnancy, including miscarriage and stillbirth, such as early abortion, late abortion, spontaneous abortion, induced abortion, therapeutic abortion, threatened abortion, missed abortion, incomplete abortion, complete abortion, habitual abortion, missed abortion, and septic abortion; ectopic pregnancy, anemia, Rh incompatibility, vaginal bleeding during pregnancy, gestational diabetes, intrauterine growth retardation, polyhydramnios, HELLP syndrome, abruptio placenta, placenta previa, hyperemesis, preeclampsia, eclampsia, herpes gestationis, and urticaria of pregnancy. Additionally, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of diseases that can complicate pregnancy, including heart disease, heart failure, rheumatic heart disease, congenital heart disease, mitral valve prolapse, high blood pressure, anemia, kidney disease, infectious disease (e.g., rubella, cytomegalovirus, toxoplasmosis, infectious hepatitis, chlamydia, HIV, AIDS, and genital herpes), diabetes mellitus, Graves' disease, thyroiditis, hypothyroidism, Hashimoto's thyroiditis, chronic active hepatitis, cirrhosis of the liver, primary biliary cirrhosis, asthma, systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenic purpura, appendicitis, ovarian cysts, gallbladder disorders, and obstruction of the intestine.

Complications associated with labor and parturition include premature rupture of the membranes, pre-term labor, post-term pregnancy, postmaturity, labor that progresses too slowly, fetal distress (e.g., abnormal heart rate (fetal or maternal), breathing problems, and abnormal fetal position), shoulder dystocia, prolapsed umbilical cord, amniotic fluid embolism, and aberrant uterine bleeding.

Further, diseases and/or disorders of the postdelivery period, including endometritis, myometritis, parametritis, peritonitis, pelvic thrombophlebitis, pulmonary embolism, endotoxemia, pyelonephritis, saphenous thrombophlebitis, mastitis, cystitis, postpartum hemorrhage, and inverted uterus.

Other disorders and/or diseases of the female reproductive system that may be diagnosed, treated, and/or prevented by the polynucleotides, polypeptides, and

agonists or antagonists of the present invention include, for example, Turner's syndrome, pseudohermaphroditism, premenstrual syndrome, pelvic inflammatory disease, pelvic congestion (vascular engorgement), frigidity, anorgasmia, dyspareunia, ruptured fallopian tube, and Mittelschmerz.

5

Infectious Disease

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

15 Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, 20 Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, 30 Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic

T03T90" T2T28860

fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific

5 embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment

10 polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial and fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following Gram-

15 Negative and Gram-positive bacteria, bacterial families, and fungi: Actinomyces (e.g., Norcardia), Acinetobacter, *Cryptococcus neoformans*, Aspergillus, Bacillaceae (e.g., *Bacillus anthracis*), Bacteroides (e.g., *Bacteroides fragilis*), Blastomycosis, Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucella, Candidia, Campylobacter, Chlamydia, Clostridium (e.g., *Clostridium botulinum*, *Clostridium difficile*,

20 *Clostridium perfringens*, *Clostridium tetani*), Coccidioides, Corynebacterium (e.g., *Corynebacterium diphtheriae*), Cryptococcus, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (e.g., *Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (e.g., *Salmonella typhi*, *Salmonella enteritidis*, *Salmonella typhi*), Serratia, Yersinia,

25 Shigella), Erysipelothrix, Haemophilus (e.g., *Haemophilus influenza* type B), Helicobacter, Legionella (e.g., *Legionella pneumophila*), Leptospira, Listeria (e.g., *Listeria monocytogenes*), Mycoplasma, Mycobacterium (e.g., *Mycobacterium leprae* and *Mycobacterium tuberculosis*), Vibrio (e.g., *Vibrio cholerae*), Neisseriaceae (e.g., *Neisseria gonorrhea*, *Neisseria meningitidis*), Pasteurellaceae, Proteus, Pseudomonas

30 (e.g., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (e.g., Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Staphylococcus (e.g., *Staphylococcus aureus*), Meningiococcus, Pneumococcus and Streptococcus (e.g., *Streptococcus*

pneumoniae and Groups A, B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye infections (e.g., conjunctivitis), uveitis, tuberculosis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., meningitis types A and B), chlamydia, syphilis, diphtheria, leprosy, brucellosis, peptic ulcers, anthrax, spontaneous abortions, birth defects, pneumonia, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory diseases, candidiasis, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections, noscomial infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Schistosoma, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium virax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be

used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage.

Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of

09882171 061801

non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

Gastrointestinal Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose gastrointestinal disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowel lymphoma)), and ulcers, such as peptic ulcers.

Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adeoncarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers, polyps of the stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum, hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, bubphrenic abscess,).

Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata*, *Echinococcus granulosus*, *Diphyllobothrium spp.*, and *T. solium*).

Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolenticular degeneration, hepatomegaly, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile

hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, 5 hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Kaposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]], peliosis hepatis, erythrohepatic porphyria, hepatic 10 porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and 15 glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic fibrosis, cyst (pancreatic pseudocyst, pancreatic fistula, insufficiency)).

Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, 20 acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

Diseases and/or disorders of the large intestine include antibiotic-associated colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous 25 adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon [Hirschsprung disease, toxic megacolon]; sigmoid diseases [proctocolitis, sigmoid neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases 30 (ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease (ulcerative colitis, Crohn's disease), intestinal atresia, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis,

amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction, impacted feces, intestinal pseudo-obstruction [cecal
 5 volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jéghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowel syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing enteropathies (intestinal
 10 lymphagiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse, rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer, Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia,
 15 gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and
 20 hemorrhagic colitis.

Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract
 25 neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms),
 30 esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, atresia, cyst, diverticulum (e.g., Zenker's diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome),

stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)),
 5 hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal diseases (appendicitis, cecal neoplasms)).

Chemotaxis

10 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then
 15 fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the
 20 number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as
 25 agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

30 A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The

binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

5 Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural
10 receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

 Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane
15 containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

 The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving
20 competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

 Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate
25 compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

 Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The
30 antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

09882171.061801

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R.

Biotechniques 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and $^3\text{[H]}$ thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the

uptake of $^3\text{[H]}$ thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of $^3\text{[H]}$ thymidine. Both agonist and antagonist compounds may be identified by this procedure.

5 In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured.

Alternatively, the response of a known second messenger system following
10 interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

15 All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from
20 suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of
25 identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

5 As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention
10 (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate
15 episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

20 By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes
25 known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a
30 non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of

benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxycetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present

invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Polypeptides of the Invention Binding Peptides and Other Molecules

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind polypeptides of the invention, and the polypeptide of the invention binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of: contacting a polypeptide of the invention with a plurality of molecules; and identifying a molecule that binds the polypeptide of the invention.

The step of contacting the polypeptide of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptide of the invention on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptide of the invention. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized polypeptide of the

invention. The molecules having a selective affinity for the polypeptide of the invention can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptide of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptide of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptide of the invention and the individual clone. Prior to contacting the polypeptide of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for a polypeptide of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the polypeptide of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound polypeptide of the invention, or alternatively, unbound polypeptides, from a mixture of the polypeptide of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction.

Such a wash step may be particularly desirable when the polypeptide of the invention or the plurality of polypeptides is bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind to a polypeptide of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R. B., et al., 1992, *J. Mol. Biol.* 227:711-718; Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, *Proc. Natl. Acad. Sci. USA* 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is

great. For example, Ecker and Crooke, 1995, *Bio/Technology* 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992, *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds a polypeptide of the invention can be carried out by contacting the library members with a polypeptide of the invention immobilized on a solid phase and harvesting those

library members that bind to the polypeptide of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

5 In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to a polypeptide of the invention.

Where the polypeptide of the invention binding molecule is a polypeptide, the
10 polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

15 Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine.

20 Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

As mentioned above, in the case of a polypeptide of the invention binding
25 molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a polypeptide of the invention binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

30 The selected polypeptide of the invention binding polypeptide can be obtained by chemical synthesis or recombinant expression.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited
 5 clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA
 10 or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300
 15 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These
 20 experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is
 25 heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA
 30 oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense

05882171.061801
 1008190 128860

RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or
5 a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard
10 in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early
15 promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

20 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA,
25 forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the
30 invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

09882171 061801
108190 "T 2128850

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of

the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide

may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to

modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to treat weight disorders, including but not limited to, obesity, cachexia, wasting disease, anorexia, and bulimia.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the
5 ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide
10 sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human
15 cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological
20 sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained
25 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

30 Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence

09882171.061801

selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least

95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise
5 a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1
10 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the
15 amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the
20 Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid
25 sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

30 Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human

09882171.061801

cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a

sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained
5 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

10 Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at
15 least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

20 Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules
25 in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in
30 Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

05682171-061801

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-

human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

- 5 In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 2, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3
- 10 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 2. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby
- 15 incorporated by reference in their entirety.

TABLE 2

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
1	HTTEZ21	11	706584	R37023, R46612, R53545, R42200, R46612, H16400, H19801, H29808, N39235, N39576, N75064
1	HTTEZ21	197	637737	R37023, R42200, R46612, R53545, R42200, R46612, H16400, H19801, H29808, N39235, N39576, N75064
1	HTTEZ21	198	319919	R37023, R42200, R46612, R53545, R42200, R46612, H16400, H19801, H29808, N39235, N39576, N75064
2	HBGBW52	199	330742	H95265, H95253
7	HCUIM14	17	330779	H92793
9	HEIAX07	201	330847	T96944
10	HSAXR76	202	330900	H14823, H24074, W15573, W31649
12	HCFAW04	204	331039	H18999, H19100, H49530, H51389
13	HLMAV65	23	646698	T56702, N32010, N32399, AA076337, AA076338, AA425636, AA424836
13	HLMAV65	206	331549	T56702, N32010, N32399, AA076337, AA076338
13	HTXEF04	207	513807	T56702, N32010, N32399, AA076337, AA076338
14	HPMFD84	208	332678	R63484, R69614, R69634, R69704, R69731, H01317, R87745, H70638, N22190, N91941,

				W24315, W46759, W47175, W49748, W89186, AA010780, AA011188, AA056460, AA149744, AA149912
15	HE6DB26	209	333590	R22155, R78386, W32024, W44434, W57584, AA054473
16	HHFFL33	26	331218	T64856, T72598, R19472, R21257, R43813, R51233, R51234, R43813, H07887, H07978, H88789, H89008, H88789, N34394, N48632, N67378, W47273, AA131246, AA131514, AA167333
21	HCFME41	211	338913	H56624, H80486, H85080, H87334, H90056, AA079169, AA079170, AA172091
23	HOSEX08,HOSEX91	212	339067	T61276, R39946, R44197, R44197, R61214, R61334, R79100, N22563, N33794, N75784
25	HEBEB69	213	340233	AA084458
26	HE6EH18	214	340242	R38946, R45145, R45976, R45976, R45145, N51406
29	HBMVK68	39	340290	R12213, T79175, T79259, R39934, R52525, R52573, H90609, N34140, N52014, N54627, AA007443, AA007495, AA075148, AA075130, AA125964
30	HMKDC66	40	340310	R72889, R87492, N59283
31	HMKCU94	215	340313	T55569, T55648, N92368, AA069305, AA098911
32	HRDEW41	42	621126	R16110, R16010, R59375, R59433, R69306, R69307, R80568, R80569, H48373, N25999, N36631, N59357, N76458, N95556, W52821, AA025244, AA026038, AA027997, AA034306, AA034307, AA043521, AA134138, AA130293, AA132093, AA132193
32	HRDEW41	216	340315	T69152, T69213, T80080, T80327, R19043, R27520, R38534, R38898, R44031, R44031, R67769, H11493, H11852, H13644, H22161, H39529, H43488, N32678, N50022, N51861, N54126, N54677, W16972, W32896, W35293, W38598, N89624, N90277, AA027830, AA027892, AA035739, AA055806, AA069223, AA078890, AA078891, AA099437, AA099478, AA101431, AA112543, AA121794, AA129629, AA136251, AA143110, AA150576, AA157125, AA158242, AA158709, AA159975, AA159976, AA160357, AA159491, AA160534, AA160629, AA165150, AA165151, AA164643, AA166799, AA169647, AA169822, AA173082, AA187009
33	HTOJN06	43	340319	T65361, R63807, R64413, H13435, H13484, N20133, W67880, AA013276, AA017015, AA027808, AA029304, AA056160
34	HBGDA21	217	340334	T51241, T51249, T51356, T51363, R11160, R11161, H45288, H51300, H97524, N72234, W40241, W40244, W45480, W45486, W46818, W60803, W60934, W95170, W95133, AA166967, AA167045, AA191454
35	HFGAK75	218	340384	R12864, R38437, R49877, R49878, R56104, R56274, R56369, R56497, R62408, R62409, R80227, R80333, H22843, H22844, N40663, N80562, W07685, AA039646, AA039645, AA084546, AA143714, AA143751, AA179782,

				AA180189, AA180499, AA181066, AA181067
36	HHPBD40	46	340399	T64787, T72540, R17957, R17989, R19224, R43447, R43480, R44487, R54708, R43447, R43480, R44487, H02892, H03792, H08062, H39801, R96240, R96296, H55925, H61904, H79118, H79209, AA004660, AA004665, AA004855, AA032203, AA032270, AA116120, AA116121, AA193583, AA193627
37	HOVCL83	47	340407	R38234, N21541, N50827
39	HBICM48	49	497206	T66994, T66995, T90274, T81704, R16104, R26725, R26952, H09075, H09424, R94094, R96182, R96183, N79107
39	HBICM48	219	340480	T66994, T90274, R16104, R26725, R26952, H09075, R94094, R96182, R96183, N79107
40	HLTCL35	50	635471	T81400, T81399, T97001, T97115, H80498, H81416, H91376, AA258096
40	HLTCL35	220	340536	T81400, T81399, T97001, T97115, H80498, H81416, H91376
41	HLHCK50	51	792650	T49685, T69050, T85769, R00655, R20782, R20827, R20891, R27806, R27898, R33703, R42007, R52046, R52047, R42007, R66604, R66605, R66641, R72373, R72374, H00427, H00428, H00955, H00956, H15928, H25414, H27488, H28652, H28790, H28764, H45792, H46560, R94564, R94565, H51645, H62694, H97650, N70289, N81184, N94729, N98562, W61307, W65372, AA009816, AA009817, AA057612, AA057696, AA078845, AA079279, AA079449, AA080946, AA129723, AA133263, AA133975, AA134086, AA134087, AA130451, AA147199, AA149934, AA148549, AA150474, AA158715, AA159354, AA159543, AA160770, AA256558
41	HLHCK50	221	340581	R33703, R66605, R72374, H00956, H15928, H27488, H45792, H51645, H97650, N70289, N81184, N98562, AA009816, AA009817
42	HRSAN45	52	340592	T86115, R16132, N78938, N94960, AA062910, AA128760, AA129755, AA133232, AA135673
43	HSNBB14	222	340597	R33866, H03918, H03919, H04645, H04665, H20263, H20335, H40675, AA015653, AA045174, AA045293
44	HMABL38	54	638246	W44379
45	HSKDK47	55	340647	R30992, N43847, N50721, N73309, W31785, W32138, W44470
46	HOSFH03	56	586413	R13900, R20375, R21746, R21791, R24287, R24396, R26219, R26218, R43762, R43762, H13483, H18658, H18745, H23200, H25669, H26256, H27373, H28341, N20036, N21618, N28857, N34176, N34653, N35351, N44596, N63461, N63485, N64276, N72203, N74518, N94754, N94784, W00446, W02907, W70310, W78207, AA056747, AA058427, AA069438, AA069465, AA082046, AA151448, AA151449, AA227737, AA228122, AA232358
46	HOSFH03	224	340661	R13900, R20375, R21746, R21791, R24287, R24396, R26219, R26218, R43762, R43762,

				H13483, H18658, H18745, H23200, H25669, H26256, H27373, H28341, N20036, N21618, N28857, N34176, N34653, N35351, N44596, N63461, N63485, N64276, N72203, N94754, N94784, W00446, W02907, W70310, W78207, AA056747, AA058427, AA069438, AA069465, AA082046, AA151448, AA151449
47	HOGAV75	57	589232	R17443, R56086, R56198, H24103, H28916, R97505, R97504, H48742, H67668, H98111, N23177, N30825, N31695, N32642, N42454, W16865, W19323, W19290, W24066, W25486, W25532, W76176, AA021599, AA069784, AA084777, AA101750, AA101662, AA148552, AA148551, AA150175, AA161441, AA164926, AA227354, AA227355, AA232813, AA463377
47	HOGAV75	225	340663	R17443, R56086, R56198, H24103, H28916, R97505, R97504, H48742, H67668, H98111, N23177, N30825, N31695, N32642, N41350, W16865, W19323, W19290, W24066, W25486, W25532, W76176, AA021599, AA069784, AA069834, AA081911, AA084777, AA101750, AA101662, AA148552, AA148551, AA150175, AA161441, AA164926
48	HFCAl74	58	340677	H49529, H49767, W90083, W90209, AA031975, AA031974, AA084096
49	HAGBI17	59	652566	T40566, R10098, T82863, T27093, H06498, H06555, H22066, H60162, H60205, H88668, H88715, N52473, N63318, N77435, N95489, W16826, W49660, W49744, W84518, AA025137, AA025153, AA037221, AA037220, AA088241, AA137103, AA150492, AA172393, AA173824, AA196791, AA235338, AA235769, AA427925
49	HAGBI17	226	340679	T40566, R10098, T82863, T27093, T27149, H06498, H06555, H22066, H60162, H60205, H88668, H88715, N25857, N52473, N63318, N77435, N95489, W16826, W49660, W49744, W84518, AA025137, AA025153, AA037221, AA037220, AA088241, AA137103, AA150492, AA156695, AA172393, AA173824
51	HPRCA31	61	581445	T66819, T66820, T85679, R13750, R13931, R25695, R38596, R53117, R53215, R83626, R83773, R89758, R89781, H55813, H60033, W69796, W69884, W73236, W73291, W94781, W95297, AA071091, AA156167, AA156502, AA223256, AA223356, AA458973, AA459189
51	HPRCA31	227	340708	T66819, T66820, T85679, R13750, R13931, R25695, R38596, R45667, R53117, R53215, R45667, R83626, R83773, R89758, R89781, H60033, W69796, W69884, W73236, W73291, W94781, W95297, AA071091, AA156167, AA156502
52	HPRCE95	62	646288	T75226, R05772, R05858, R35378, R44456, R51239, R51240, R44456, R67589, R99125, R99667, H80457, H81377, H90424, N22077, N75320, W04771, W40159, W45375, W57547, W78826, W80725, AA001824, AA026071,

				AA026072, AA099306, AA099816, AA160137, AA169306, AA171668, AA173427, AA193330, AA194113, AA430050, AA430259
52	HPRCE95	228	340712	T75226, R05772, R05858, R19669, R35378, R44456, R51239, R51240, R44456, R67589, R99125, R99667, H62128, H80457, H81377, H90424, H90480, N22077, N75320, W04771, W40159, W45375, W57547, W78826, W80725, AA002028, AA001824, AA026071, AA026072, AA099306, AA099816, AA160137, AA169306, AA171668, AA173517, AA173427, AA193330
53	HHTLC66	63	340716	T93117, T93217, R18513, R35994, R36368, R54402, R54501, R78179, H21223, H30750, H43172, R90745, R90785, H94364, N51284, W02098, W32304, AA017239, AA017330, AA025447, AA025448, AA083964, AA112163, AA127498, AA127520, AA147192, AA158669, AA158670, AA158668, AA158671, AA160391, AA181678, AA190725, AA190909, AA191485
54	HMADJ02	64	665037	T55474, T85674, R32236, R35180, R81909, H18878, H20360, H22508, H41039, H46678, R89209, R99132, H58424, H69768, H72482, N42965, N44128, N46497, N57338, W25680, W25676, W52136, W67531, W92351, AA018287, AA018347, AA127971, AA151257
54	HMADJ02	229	340733	T55474, T55401, T60074, T91252, T85674, R32185, R32236, R34238, R35180, R35565, R50840, R81805, R81909, R82743, H18878, H18879, H20171, H20360, H22469, H22508, H29277, H40286, H41039, H41469, H42928, H46679, H46678, R89101, R89209, R99132, H48432, H58424, H58425, H69768, H72482, H72883, N30666, N32244, N36122, N36966, N38925, N42965, N44128, N46497, N47999, N51206, N57338, N69681, N94289, N98733, W02508, W02840, W25680, W25676, W30822, W31172, W37751, W37750, W52136, W67532, W67531, W92351, AA001534, AA018287, AA018347, AA018348, AA025474, AA025393, AA054937, AA055147, AA084018, AA112174, AA127920, AA127971, AA127062, AA125939, AA142846, AA151257, AA151258
55	HPRCU93	230	340745	AA044652
56	HSAXS65	231	340750	R39323, H05070, H41666, N31947, N42783, N63271, N90340, AA044807, AA044784, AA099107, AA131748, AA131836, AA143351
57	HKTAG35	67	750765	T63010, T63155, N80942, W78114, W79821, AA027951, AA027916
57	HKTAG35	232	422830	T63010, T63155, N80942, W78114, W79821, AA027951, AA027916
57	HMEFX42	233	340769	T63010, T63155, N80942, W78114, W79821, AA027951, AA027916
58	HHFHN61	68	340778	R39487, R39488, R70790, R70844, H65872, H65871, N35912, W16998, AA007279, AA165622, AA165658, AA193643
62	HATDT67	72	637512	W95483, W95374, AA464846

62	HATDT67	236	340872	W95483, W95374
64	HMWEX24	74	636164	T63776, T63811, R23451, R26050, R54566, R71431, H14303, H21785, H21786, H25266, H30712, H41912, H44413, R88668, R88667, H83339, H99419, N20174, N29075, N33561, N33856, N34443, N37060, N42851, N46372, N51305, N62831, N67782, N72280, W01367, W03024, W03584, W20085, W72602, W76567, N91480, AA036951, AA036992, AA069144, AA069232, AA074084, AA075977, AA127754, AA179521, AA179344, AA179568, AA179576, AA236305, AA252367, AA252744, AA256845, AA256211, AA256433, AA262880, AA418903, AA421392
64	HMWEX24	238	340878	T63776, T63811, R23451, R26050, R54565, R54566, R71431, H83339, H99419, N20174, N29075, N34443, N37060, N42851, N46372, N51305, N62831, N72280, W01367, W03024, W20085, W72602, W76567, N91480, AA074084, AA075977, AA179521, AA179344, AA179568, AA179576
65	HSGBA84	75	340917	T70586, T70850, H45252, H45316, W94688, W95194
66	HTOCD52	239	340926	R96763
68	HKIXR69	78	498091	T27138, T27139, R77447, R77540, H13397, H24086, H24199, N93267, W25026, AA033849, AA034353, AA041406, AA236384, AA262937
68	HKIXR69	240	340959	T27138, H24086
69	HETGJ09	79	340981	H15437, H98603, N36770, N45930, N49865, N49875, N51072, N51762, N70334, W94942, AA056608, AA137182
70	HOBNC61	80	340986	R41269, R41269, R69456, R70443, H38923, N59126, N59131, N75166, N75171, W15406, W39519
71	HFFAH94	81	341007	T40317, T51739, T53472, T46971, T47506, T48920, T48921, T49015, T49016, T49175, T49298, T49299, T49695, T49701, T49997, T50027, T50057, T52080, T52658, T52659, T53330, T53331, T53627, T53663, T53758, T54085, T54152, T54175, T63372, T75017, R06020, R23398, R33890, R63243, R63756, R75655, R75765, H00189, H00241, H09883, H17683, H42345, R86098, R87290, R89690, R91869, H52013, H64772, H73693, H75892, H84586, H89190, N29678, N32054, N42026, N47931, N56634, N57153, W07751, W07769, W20151, W31959, W38588, W44842, AA001770, AA058334, AA081895, AA083423, AA083636, AA083919, AA084837, AA085235, AA115438, AA122406, AA133479, AA132347, AA136452, AA146919, AA147421, AA151894, AA152160, AA156975, AA157489, AA157487, AA157934, AA159425, AA159732, AA164481, AA165483, AA172314, AA179193, AA181685, AA187840, AA188093, AA186492, AA186783, AA186793, AA186918, AA186935, AA187007, AA187053

2025年12月22日

73	HSQEL25	83	639101	R85294, AA150488
73	HSQEL25	241	781516	R85294, AA150488, AA186761
73	HSQEL25	242	341015	R48156, R72687, R85294, AA150488, AA187629
74	HEBEG68	84	341023	T67556, T69552, T84413, T85269, T96893, R46761, R46856, H01797, H69563, H70019, H84661, H85196, H92136, H92137, H93474, H93473, H96838, N51712, N51801, N62956, N63622, N75603, N80289, N95238, W00738, W05621, W07127, W21067, W94925, W92196, W95269, W95270, AA179187
75	HBIAB39	85	667525	T64303, T64688, R66180, R67060, H19829, H19859, H29834, H87148, H87649, H89959, N35923, N36775, N42201, N45936, N72214, W00490, AA036653, AA037218, AA064615, AA064673, AA082867, AA085339, AA121400, AA242888, AA252242, AA262055
75	HBIAB39	244	341025	T64303, T64688, R66180, R67060, H19829, H19859, H29834, H87649, H89959, N35923, N36775, N45936, W00490, AA036653, AA037218, AA064615, AA064673, AA082867, AA085339, AA121400
76	HTXDU73	86	341029	T66720, R16261, H84276, H85593, AA148543, AA156634
77	HOEAS24	245	634500	T67464, T69497, T86573, T86750, T87354, T78865, T95251, T95252, R51805, R51806, R60720, R60721, R61717, H25779, H61825, H61824, N48718, W44894, AA010204, AA010241, AA043867, AA044312, AA460349, AA460778
77	HOEAS24	246	341034	T67464, T69497, T86573, T86750, T87354, T78865, T95251, T95252, R51806, R60720, R60721, R61716, R61717, H25779, H61825, H61824, N48718, W44894, AA010204, AA010241, AA043867, AA044312
78	HTEIY30	88	341110	R12814, R20726, R40236, R20726, R40236, H09494, H09495, N21561, N31709, N31708, N42363
79	HSKNE46	247	341121	R74441, R86080
80	HPMFL27	90	341124	T48869, T48870, T49904, T53681, R25109, R25110, R25671, R27528, R31935, R31981, R33395, R36105, R36203, R68383, R68588, R73906, R73999, R75632, R75732, R75957, R78948, R79050, H02580, H02668, H03502, H03868, H03869, H12451, H12498, H12839, H51263, H51833, N50695, N58781, N77468, AA035486, AA035018, AA147247, AA147933
81	HMWDN32	91	341126	R02692
82	HPRAX55	248	341130	R46337, R54837, R72518, H41918, H41962, H58330, H58719, H82709, N32438, N72856, AA004403, AA053671, AA164257, AA164368
83	HHFFW36	93	341162	T65334, R06680, R32977, R32978, R33001, R33000, R64154, R64264, R66122, R67730, H01946, H09092, H09441, H12088, H12089, H39822, H88622, H88622, H99675, N28430, N33410, N44873, N72029, N99481, AA128356, AA128519, AA192635, AA192784, AA192783
84	HE2PL77	94	341180	T65492, T69710, T70367, R14102, H13508,

				H24901, H30495, R84506, R85296, R92833, H53504, H53874, H58116, H64947, H64948, H83182, H88075, H88075, N20214, N29281, N33314, N44846, N67850, N74617, W00890, W03453, W73139, W73119, AA039436, AA039435, AA143645
85	HSDFV29	95	726747	T93478, T96318, H65329, H65527, N20594, N24306, N26872, N28990, AA040017, AA056928, AA057074
85	HSDFV29	249	426853	T93478, T96318, R02524, H65329, H65527, N20594, N24306, N26872, N28990, N53061, N73248, W93593, AA010653, AA037624, AA040017, AA056928, AA057074, AA134768
85	HCQAV53	250	341197	T93478, T96318, R02524, N20594, N26872, N53061, N73248, W93593, AA010653, AA037624, AA040017, AA057074, AA134768
86	HTPEG42	96	655475	R68003, R68002, H92758, W20199, W38589, W74674, W74780, N90213, N90553, AA043957, AA043823, AA126598, AA157016
86	HTPEG42	251	341206	W38589, W74674, W74780, N90213, AA043957, AA043823, AA126598, AA157016
87	HLHDR57	97	341213	R60239, R60474, N70344, W04155, AA037509, AA039803, AA054769, AA112956, AA172011, AA191112
88	HAUAV32	252	341214	N95062, W19506, W38899, W52820, N89912, AA035640
89	HNEBI60	253	341219	R08642, R08738, T78434, T96975, T97087, R27984, R46404, R46404, R63138, H21791, H21792, H26134, H26399, H41136, H41403, H43201, H43240, R98484, R98531, H56649, H56726, H78891, N54287, AA150592
90	HSJC16	100	341227	T63745, T74291, T77596, T91992, T92076, R01666, R02606, R30785, R50817, H14952, H17656, H17773, R88235, R88234, R96151, W37867, W37858, W52057, W60602, AA126008, AA126114, AA134190, AA134208, AA173351
91	HTSEL31	101	341228	T77424, T86561, T86739, T86066, R17360, R38366, R42711, R44414, R42711, R44414, R69488, R70382, R75611, R75701, H26361, H27471, H40753, AA074108, AA074906, AA074981, AA133487, AA148391, AA148392, AA187336, AA188462
92	HAUBL57	254	630116	T87331, T87432, R16493, R16793, R20740, R22276, R22746, R34517, R39968, R52851, R20740, R39968, H30186, H42692, H39131, H43926, H44586, H46389, R99312, R99404, H84964, N41538, N42109, N98255, W01018, W19986, W81009, W99356, W99386, N90413, AA009837, AA010386, AA010322, AA019377, AA033587, AA056148, AA079689, AA081447, AA100074, AA100159, AA114264, AA134510, AA134519, AA157284, AA157365, AA161199, AA161200, AA181973, AA187561, AA227076, AA227062, AA232373, AA233577, AA233594, AA256297, AA256403, AA417901, AA418057, AA430035, AA430244

97	HAQBF73	260	341243	T62952, T92192, T92225, T77665, R11188, T81487, T81700, R00485, R00584, R02320, R02422, R20298, R22227, R23678, R40975, R42968, R43639, R42968, R40975, R43639, R67269, R68907, H02952, H04250, R93173, R93265, R94343, H56904, H56992, H83900, H97837, N29328, N30433, N42347, N47549, N47550, N47895, N49858, N51150, N56938, N57020, N59159, N63879, N66463, N71798, N75895, N76091, N77882, N92023, W04435, W05139, W05635, W19637, W57588, W73239, W73294, W92204, W92205, N89882, AA074753, AA074836, AA171536, AA192742, AA192815
98	HAQBT94	108	341244	R00206, R01689, R02630, N30938, N34891, N99874, W25308
99	HETHE07	261	634935	R24661, R25164, R31031, R31521, R36666, R39748, R41825, R41825, R76400, R76739, H03481, H03576, H29806, H73483, H75574, H96448, H96862, W67151, W67394, W81230, W81266, AA040399, AA054370, AA054507, AA074719, AA075225, AA076083, AA076164, AA115461, AA115066, AA165265, AA165266, AA164408, AA164409, AA164486, AA164492, AA164493, AA164487, AA169668, AA169281, AA169420, AA173414, AA173443, AA173439, AA173493, AA173499, AA173504
99	HETHE07	262	341247	R24661, R25164, R31031, R31521, R36666, R39748, R41825, R41825, R76400, R76739, H03481, H03576, H29806, H73483, H75574, H96448, H96862, W67151, W67394, W81230, W81266, AA040399, AA054370, AA054507, AA074719, AA075225, AA076083, AA076164, AA115461, AA115066, AA165265, AA165266, AA164408, AA164409, AA164486, AA164492, AA164493, AA164487, AA169668, AA169281, AA169420, AA173414, AA173443, AA173439, AA173493, AA173499, AA173504
100	HLQAB52	263	341250	H43350, N27368
101	HCRAM28	111	341252	T65107, T97334, R53476, H09583, H30548, H71011, H95075, N29391, N30834, N41362, N74154, N75079, N92831, N95057, W19524, W72254, AA025307, AA024537, AA025223, AA160270, AA160271, AA167140, AA167276, AA188817
101	HIBEK16	265	638538	T65107, T97334, R53475, R53476, H09583, H30548, H71011, H95075, N29391, N30834, N41362, N57317, N74154, N75079, N92831, N95057, W04321, W19524, W25059, W72254, AA025307, AA024537, AA025223, AA160270, AA160271, AA167276, AA188817
102	HE2BG03	266	341253	N49408, N49498, N55047, W03070, W67229, W67339, W77952
103	HEBDJ82	113	341263	R11788, T96282, T96281, R18963, R33113, R33261, R37707, R43974, R52405, R52406, R52462, R52463, R43974, R55831, R55917, R56079, R56190, R63506, R63551, R66072,

				R67672, H04652, H04674, H14855, H88794, H89018, H88794, N29740, N57160, W45643, W45596, W69792, W69880, AA043192, AA043594
104	HCUBC79	114	639176	R26344, R66398, R67132, R67854, R68609, R69202, R74181, R74266, H13077, R93558, H87000, H95448, H96357, W39616, N91087, AA134291, AA176656, AA176854, AA188764, AA256577
104	HCUBC79	267	587008	R26344, R66398, R67132, R67854, R68609, R69202, R74181, R74266, H13077, R93558, H87000, H95448, H96357, W39616, N91087, AA134291, AA176656, AA176854, AA188764, AA256577
104	HCUBC79	268	341271	R26344, R66398, R67132, R67854, R68609, R69202, R74181, R74266, H13077, R93558, H65931, H87000, H95448, H96357, W39616, N91087, AA134291, AA176656, AA176854, AA188764
105	HSVAF07	115	647593	T49542, T49543, N79115, N93812, W44963, W65318, W65317, AA005085, AA099521, AA223969, AA426123, AA425831, AA428347
105	HSVAF07	269	637678	T49542, T49543, N42222, N62353, N79115, W44963, W44467, W65318, W65317, AA005085, AA099521, AA099520, AA223969
105	HSVAF07	270	341274	T49543, N42222, N62353, N79115, N93812, W44428, W44467, W65318, AA005085, AA099521, AA099520
106	HT3AM65	271	550213	R50060, H22175, R84872, R85806, R86903, R86915, H54447, H54537, H71458, H71587, N52360, N64314, N75131, W52304, W56703, AA025637, AA025638, AA033583, AA033584, AA035172, AA043426, AA043427, AA148635, AA148636, AA164764, AA164765, AA215808
106	HT3AM65	272	341277	R50060, H22175, R84872, R85806, R86903, R86915, H54447, H54537, H71458, H71587, N52360, N64314, N75131, W52304, W56703, AA025637, AA025638, AA033583, AA033584, AA035172, AA043426, AA043427, AA148635, AA148636, AA164765
107	HE6DK18	117	341297	R01532, N41434, N70473, N70471, N72861, W03083, W03834, W45060, W45070, W48640, W51956, N91018, AA039299, AA039300, AA046006, AA046007
108	HEBEK93	118	656435	T63347, T63427, T63669, R39259, R68780, R70392, R73655, R73737, R80228, N26735, N27000, N30045, N40122, N62562, N63462, N79017, N95323, N99129, W19257, W24778, W42778, W42893, W74164, W76535, AA025427, AA037007, AA083212, AA083311, AA099834, AA115588, AA115078, AA135669, AA135684, AA143000, AA159331, AA159430, AA171693, AA171808, AA173590, AA173737, AA173920
108	HEBEK93	273	341298	T63347, T63427, T63669, R39259, R68780, R70392, R73655, R73737, R80228, R80334, N26735, N27000, N30045, N40122, N62562,

				N63462, N79017, N95323, N99129, W19257, W24778, W42778, W42893, W74164, W76535, AA025427, AA037007, AA083212, AA083311, AA099304, AA099834, AA115588, AA115078, AA135669, AA135684, AA143000, AA159331, AA159430, AA171693, AA171808, AA173590, AA173737, AA173920
109	HJPCM10	119	671656	T52165, T52241, T90189, T84079, R32492, R32602, H57835, H86933, H86932, N36854, N58903, N66193, N66878, N93552, N93556, N95615, N99190, AA043182, AA043183, AA042984, AA243295
109	HJPCM10	275	341315	T52165, T52241, T90189, T84079, R32492, R32602, H57835, H86933, H86932, N36854, N58903, N66193, N66878, N93552, N93556, N95615, N99190, AA043182, AA043183, AA042984, AA043039
110	HSXBL78	120	341338	T74710, T75297, R11916, R13124, R14338, R25727, R39185, R40603, R40664, R43583, R40664, R43583, R40603, R62471, R75755, H05460, H07011, H10315, H10314, H27091, H29792, H29791, R95668, N29515, N57459, W86201, W86202, AA082028
111	HOEAW81	121	638133	T83147, T84144, H87571, H91807, N30742, N41841, N94464, N94563, W15644, W30743, W30854, W31005, AA081589, AA115690, AA133509, AA134307, AA134308, AA134315, AA134316
111	HOEAW81	276	341360	T89384, T90621, T83147, T84144, H87571, H91708, H91807, H92083, N30742, N41841, N80826, N94563, N98724, W15644, W30743, W30854, W31005, AA081589, AA084667, AA115690, AA133509, AA134307, AA134308, AA134315, AA134316
112	HOEAP41	122	341363	R98994, N51380, N78067, W19481, W42782, W42897, W52206, W69436, W69519, W73362, W73439, W94387, N90296, AA037138, AA046403, AA069722, AA082067, AA082024, AA157654, AA157755
113	HEAAR60	123	667582	T62691, T62842, T81833, T99606, N24671, N35462, W90766, W90699, AA188889, AA189105, AA460709, AA460883
113	HEAAR60	277	341368	T62842, T81833, T99606, N24671, N35462, W90766, W90699, AA188889, AA189105
114	HTXGS75	124	341369	T47597, T47598, T90304, T82977, T99735, T99736, R17706, R38999, R42722, R42786, R42722, R42786, H85229, H95203, N24689, N29773, N30154, N35767, N36667, N57192, N70880, N76099, N98288, W05394, W06883, W19087, AA056547, AA056668, AA121234, AA121235, AA149160, AA149159, AA182535, AA188679, AA191046
115	HOVBA03	125	638141	H83533, AA143176, AA159616, AA165673, AA159698
115	HOVBA03	278	341382	H83533, N47656, N47744, AA143176, AA159616, AA165673, AA159698

117	HBMUW78	127	656003	T53926, T92254, T92553, T92625, T93804, T93855, R32495, R32605, R40235, R40235, R63831, R64443, H13418, H13461, H22555, H24408, R94238, R94335, H57449, H59016, H59017, H64002, H64051, H70912, H70995, H78449, H70912, N20822, N25219, N30547, N39398, N44197, N62369, N62884, N62878, N77785, N77791, N79139, W70226, AA059074, AA133688, AA169568, AA169873, AA195055, AA195678, AA195679, AA213529, AA214545
117	HBMUW78	280	341396	T92254, T92553, T92625, T93804, T93855, R32495, R32605, R40235, R40235, R63831, R64443, H13418, H13461, H22555, H24408, R94238, R94335, H57449, H59016, H59017, H64002, H64051, H70912, H70995, H78449, H70912, N20822, N25219, N30547, N39398, N44197, N62369, N62884, N62878, N77785, N77791, N79139, W70226, AA059074, AA133688, AA169568, AA169873
118	HASAS24	128	341406	T52688, T52687, R50972, R59542, H00394, H11046, H71487, H71487, N36814, N46032, N69321, AA099572, AA101976, AA150971, AA151080
119	HSIDN55	129	341411	T47196, T90531, T81327, T83066, R02389, R49397, R49397, R73170, H69590, N46989, N49400, N49489, N51462, N57532, N66084, N79047, N92406, N98473, W24197, W24631, W55871, W56299, W65365, W72845, W76092, AA029710, AA029642, AA039563, AA039564
120	HGBGZ64	130	341422	T77791, R10242, T84472, R89115, R94929, R94990, R95013, N51474, N53615, N80601, W42706, AA040741, AA040742, AA043440, AA053029, AA084287, AA084288, AA151396, AA151397
121	H6EBJ64	131	637481	R51043, R51157, R79165, R79266, H05444, R91173, R91222, R91701, R92139, R99529, H63402, H63455, H65251, H65864, H65874, H69798, H70976, W42837, W84415, AA029221, AA029222, AA031485, AA031464, AA037310, AA045771, AA045962, AA193397, AA253178, AA253078
121	H6EBJ64	281	341423	R51043, R51157, R79165, R79266, H05444, R91173, R91222, R91701, R92139, R99528, R99529, H63402, H63455, H65251, H65252, H65863, H65864, H65873, H65874, H69798, H70976, W42747, W42837, W84415, AA029221, AA029222, AA031485, AA031464, AA037310, AA045771, AA045962, AA193397
122	HOECP43	132	341430	H20033, H20113, H50094, H50095, N44916, N66466, W81365, W81624, AA131047, AA130966
123	H2CBV31	133	341436	T94047, T94797, T75357, R10575, T80348, T80455, T81292, R01189, R01300, R17953, R20854, R21132, R34459, R38683, R39040, R39061, R42034, R43442, R46727, R42034, R43442, R46727, R67985, H19293, H19292, H78408, H78506, N39442, N48310, N48554,

				N49035, N94078, W02832, W30814, W49543, W49774, AA039485, AA039484, AA062802, AA082406, AA088216, AA189147
124	HPCAD23	134	341438	T39517, T40647, R08890, T79580, T79658, R39186, R44549, R44549, H29275, H29360, H68472, N29403, N36337, W92928, W92929, AA012938, AA012885, AA017220, AA017313, AA037431, AA037247, AA037535
125	HSPAG15	135	341444	N24480, N35123, N62648, N68160, N80371, N80618, AA036900
126	HELGH31	136	341446	R66409, R67143, R77266, R77313, H01414, H01413, H98804, H99892, N24566, N25024, N30234, N34009, N57126, N66135, N66161, N98658, N98686
127	HUSHH48	282	341470	R13839, R36985, H03004, H05634, H12993, H50184, H99245, N26368, N27512, N29876, N42211, N42520, N58934, N64797, N73798, N75644, N95515, N99424, W95429, W95556, AA031322, AA031321, AA035435, AA035436, AA101558, AA101559, AA179484, AA179505, AA179536, AA179525, AA189168
128	HLYAU95	138	341473	T70334, T70418, R32765, R62187, H01280, H05481, H27058, H61678, H61677, H73249, H98909, N22192, N24242, N32166, N32167, N32933, N71233, W01111, AA028126, AA044276, AA044252, AA047430, AA047469
129	HHSCV65	139	341476	R55035, R55065, R59065, R59066, H11375, H19623, H19622, H22362, H22393, H40210, H40211, H46219, H46469, H47010, N41006, N45484, N47600, W06944, W86913, N90740, AA133763, AA133764
130	HTTAD57	140	341482	R62307, H98858, N36520, N50449, N52248, N62504, N62512, N62869, N63975, N66790, N67455, N75852, N77775, N79652, W07436, W60913, W68604, W68801, W69914, W70179, W73347, W73467, W79509, W79608
132	HEBFU93	283	341488	R59976, H39102, R85866
133	HSGSC60	143	341490	T64219, T64405, R36668, R39750, R71095, R71442, H56180, H56396, H99262, N40260, N42314, N80584, W07706, W37443, W37568, W37623, W37624, W73084, W73188, AA082510, AA099421, AA100989, AA134925, AA135041
134	HPMGD24	144	341495	T55461, T75312, T75315, R07965, R35914, R38680, R49434, R49434, H24097, H29201, H29202, H51145, N40044, N49410, N49500, N50862, W74365, W79169, AA031988, AA037809, AA037810, AA133953, AA134020, AA148740, AA148739, AA181720, AA190790, AA190382
135	HPTVC60	145	592550	R17247, R35296, H60973, W44347, AA010018
135	HPTVC60	284	341499	R17247, R35296, H60973, W44347, AA010018
136	HSKNE18	146	615251	H92812, N39336, N45183, W05346, W24987, W63719, W67309, W67213, AA423922
136	HSKNE18	285	341502	H92812, N39336, N45183, N95495, W05346, W24987, W63719, W67309, W67213
137	HMWIF35	147	611997	T78146, R61278, R61279, R73549, H43446,

				H43445, H78315, H98875, N23168, N25102, N33160, N42291, N57672, N57687, N59658, N68645, N69875, N77530, N91661, N98635, W52629, W52630, W56233, W88468, W89033, AA420965, AA421063
137	HMWIF35	286	341506	T78146, R61278, R61279, R73549, H43445, H78315, H98875, N23168, N25102, N33160, N42291, N57672, N57687, N59658, N68645, N69875, N77530, N91661, N98635, W52629, W52630, W56233, W88468, W89033
138	HMWGI25	148	341507	T99927, R00277, R62966, R63020, H02510, H02511, H61375, H62397, H83984, N53025, N59209, N75235, W67237
139	HSKGF03	149	668029	H20149, H20243, R94133, H93190, N28258, N30555, N32545, N32551, N41895, N52973, N57208, W02873, W03036, AA025259, AA025337, AA127243, AA126526, AA148192, AA243272, AA418257
139	HSKGF03	287	645277	H20149, H20243, R94133, H93190, N30555, N32545, N32551, N41895, N57208, W02873, W03036, AA025337, AA127243, AA126526, AA148192, AA243272, AA418257
139	HSKGF03	288	341510	H20149, H20243, N28258, N30555, N32545, N32551, N41895, N52973, AA025259, AA025337, AA148192
140	HMSKE75	150	341517	T39685, T58334, T58375, T95040, T95136, R01008, R01009, R01079, R01806, R32520, R32634, R62417, R62608, R62657, H21569, H50551, H50552, H97933, N45317, N54767, N62531, N71780, N79429, W60291, W60382, W99324, AA039317, AA039237, AA126906, AA127110, AA133949, AA133950, AA150269, AA150371, AA159341, AA159411, AA166691, AA166802
141	HCMSH30	T51	645267	T52564, T55946, T73366, W05488, W21489, W23461
141	HCMSH30	289	341524	T48350, T48349, T52564, T55946, T57088, T57193, T73366, T85101, T97261, T97372, R00232, R00339, R34368, R34486, R36253, R36254, R82314, R82315, H13839, H13885, H22323, H22324, R91853, R92692, N22767, N30057, N30064, N30060, N40765, N40768, N70641, N71006, N71091, N76132, N76459, N91781, W01941, W05460, W05488, W20019, W21489, W23461, W31478, W31481, W32000, W32023, W32273, W44853, W72460, W76518, AA001714, AA001805, AA005320, AA004243, AA010720, AA011167, AA024893, AA024894, AA025007, AA024806, AA025085, AA027095, AA044819, AA044844, AA056733, AA058387, AA099737, AA099736, AA134033
142	HTWCB92	152	341529	R12145, R15078, R59983, R67165, N50425, N50481, N58860, N66880, N72725, W19898, N91244, AA023009, AA022475, AA098971, AA099874, AA131860, AA131754, AA182936, AA181586

T08T90" T228860

143	HBMDM46	153	669036	T89546, T79378, T83501, T96827, R48892, H47139, H67305, H83557, H92560, N33828, N34173, N35285, N39763, N44626, N56596, W03073, W07402, AA156699, AA236327, AA253287, AA417777
143	HBMDM46	290	341532	T89457, T79808, T83354, T96828, R48785, R78498, R78548, H00257, H00258, H47139, H66023, H68516, H83449, H92561, N24951, N25572, N26320, N29642, N32983, N32991, N39763, N56596, N57800, N66437, N80274, W85944, AA041383
144	HFAMG13	154	341533	T64876, T90951, T85837, R17002, N59579, N73645, N75336, W21293, W95777, AA025785, AA147507
145	HFXHL79	155	638175	R13316, H08897, R98996, W07340, AA131594, AA157620, AA180960
145	HFXHL79	291	341534	R13316, H08817, H08897, R98996, W07340, AA131594, AA157620, AA180960
146	HSNAK17	156	582244	T53909, R10445, T85536, R72952, H15770, H62143, W42808, AA031603, AA031637, AA182758, AA232173
146	HSNAK17	292	341541	T53909, R10445, T85536, R72952, H15770, H62143, W42808, AA031603, AA031637, AA182758
147	HCFBC03	157	635552	R32283, R32295, R32329, R32338, R53752, R53753, R66100, R67703, H42383, H43148, W90075, AA001927, AA424984
147	HCFBC03	293	341543	R32283, R32295, R32329, R32338, R53752, R53753, R66100, R67703, H42383, H43148, W90075, W90193, AA001927
147	HSJAP03	294	464384	R32283, R32295, R32329, R32338, R53752, R53753, R66100, R67703, H42383, H43148, W90075, AA001927
148	HSKGO26	158	341548	T54320, T90772, T83224, T83638, T83802, T84784, R01903, R01952, R61603, H03357, H04040, N44934, N71086, N71095, W05465, W38751, AA037109, AA081457, AA081456, AA160292, AA160293
149	HCQAV96	159	341554	T51727, T51937, R01243, R01357, R25494, R45561, R45561, R71014, H13671, H13718, H17162, H17947, H38744, H89126, H89233, H89126, H98057, N45220, N50863, N64600, N79925, W39199, W47233, N90258, AA028057, AA028058, AA084220, AA084221
150	HSNCC16	160	341568	T52645, T56803, T77815, R19061, R44103, R48608, R48711, R44103, R76418, R76417, H21046, H50569, H62124, H88678, H96335, N28505, N31055, N35202, N36658, N39983, N44201, N75851, N95279, W24554, W38410, W39503, W46946, W47243, W47289, W56401, W60889, W94931, W92202, AA082353, AA100058, AA102513, AA115079, AA115191, AA115928, AA115927, AA121322, AA132576, AA132889, AA135237, AA143499, AA156981, AA160994, AA167060, AA167165, AA167318, AA181572, AA186382

"003790" T228860

151	HTLEF62	295	341595	R15404, AA047517
152	HTLAD94	296	341646	R06128, R16166, R15426, R17678, R18984, R26888, R27120, R35870, R35871, R44869, R51276, R44869, R66882, R67967, H13315, H22113, H27381, H28345, H37951, H38579, R93605, R97908, R97907, H53653, H61431, H61432, H62657, H63776, H63826, H65287, H65810, H89508, H89654, N29760, N57179, N74909, W23437, AA015926, AA026270, AA026558, AA045484, AA046516, AA055479, AA070895, AA070985, AA086047, AA086129, AA102052, AA102053, AA100978, AA128868, AA133807, AA133806, AA134215, AA134849, AA152266, AA150204, AA171825, AA173393, AA173394, AA182536, AA182582
153	HTSFQ12	163	341653	T78257, T81592, T83408, T98857, T99453, R24503, R24602, R34836, R34932, R52437, R52501, R52500, H10205, H12773, H12827, H20540, H20632, H40525, R92740, H51257, H51827, H78114, H78113, H93565, N30243, N30795, N41570, N57137, N67626, N71081, N75985, N76340, W01320, W04402, W04410, W52430, W52471, W61358, W60048, AA040527, AA040528, AA044739, AA044794, AA133224, AA130908, AA130822, AA142867, AA151675, AA151755
154	HE6FL83	164	616853	T73457, T73524, T65316, T65344, R21668, R24170, R46558, R52243, R52244, R46558, H10183, H19602, H20914, H20913, H23732, H23751, H29842, H29931, H30765, H41436, H41563, H38484, R84972, R87333, R88049, R90853, N59099, N66303, N73535, N92979, W23841, W25041, W40499, W45502, W56621, W56651, W67369, W67629, W72130, W77854, W94427, W92638, AA043732, AA055978, AA056006
154	HE6FL83	297	341654	T73524, T65316, T65344, R21668, R24170, R46558, R52243, R46558, H10183, H20914, H20913, H23751, H29931, H30765, H41436, H41563, H38484, R84972, R87333, R88049, R90853, N59099, N92979, W23841, W25041, W40499, W45502, W56621, W67369, W67629, W72130, W77854, W94427, W92638, AA043732, AA055978, AA056006
155	HTXFJ55	165	670981	T49430, T92075, R00570, R00674, R12889, R16317, R17166, R17919, R18012, R21041, R34226, R35552, R41283, R43263, R41283, R43263, H10590, H15663, H17419, H17418, H60450, H60497, H83595, N31466, N44274, N51210, N72512, W38423, W40215, W44751, W52648, W80752, W80840, W85690, W85743, AA009995, AA009996, AA052977, AA053566, AA053470, AA065021, AA069177, AA069242, AA076347, AA113353, AA113434, AA134898, AA223817, AA243151
155	HTXFJ55	298	341661	T49430, T39608, T39610, T40697, T49431, T91991, T92075, R00570, R00674, R12889,

				R16317, R17166, R17919, R18012, R21041, R34226, R35552, R41283, R41394, R43263, R41283, R41394, R43263, R69121, H10384, H10590, H15663, H17419, H17418, H60450, H60497, H83595, H95242, N31466, N44274, N51210, N72512, W38423, W40215, W44751, W45437, W52648, W58732, W67761, W67741, W72214, W74143, W77958, W80752, W80840, W85690, W85743, AA009995, AA009996, AA052977, AA053566, AA053470, AA065021, AA069177, AA069242, AA076347, AA113353, AA113434, AA134930, AA134898, AA172161, AA172312
156	HJPCJ76	166	341664	T39919, T60946, T72529, T73752, R09799, R11461, R12389, R12763, R20500, R45320, R45320, R60969, R61636, R70489, R70579, R77628, R77629, R99728, H59663, H85076, N21970, N21980, N44811, N50899, N51453, N52600, N53595, N59331, N71174, N71184, N77139, N78503, W05163, W57664, W72044, W76396, AA007568, AA007569, AA021623, AA021217, AA035589, AA046604, AA046719, AA059114, AA059172, AA062948, AA127212, AA127195, AA135430, AA158353, AA176606, AA176879
157	HLTED27	299	341672	R14927, R19442, R20366, R38545, R39202, R43752, R44690, R51296, R51408, R43752, R81517, R81767, H10945, H15061, H15997, H16577, H16621, H44519, H53352, H53396, H68444, H99530, H99531, N29833, N47446, N47447, N49036, N57251, N64206, N64268, N66379, N92787, N94418, N95423, N98459, W24035, W30978, W32201, W32256, W56159, W58059, W58387, W60462, W94562, W92646, N90558, AA026570, AA031679, AA031680, AA031834, AA031835, AA043396, AA045186, AA046907, AA055036, AA055153, AA056450, AA074783, AA074784, AA074607, AA075380, AA075429, AA084527, AA099356, AA101888, AA121182, AA121222, AA127716, AA127771, AA127762, AA149004, AA149005, AA165300, AA165299, AA165450, AA169451, AA171961, AA171746, AA188146, AA188223
158	HMKBA64	168	341674	T96889, T96890, T97468, T97469, R97096, H64964, H93009, H94235, N47960, N49171, N59386, N77074, W01926, AA035208, AA035209, AA040430, AA040431, AA040644, AA045294, AA045521, AA058688, AA088175, AA099553, AA101990, AA160278, AA160279
159	HNFIP24	169	351534	H04128, AA099288, AA101983
160	HCELB21	170	670664	R11975, R25294, R39817, H08811, H08888, H12161, H62686, H73328, H78750, H79523, H90226, H90880, N72184, W00465, W38433, W44822, AA016301, AA033738, AA033739
160	HCELB21	300	351542	R11975, R25294, H08811, H08888, H73328, H79523, H90880, N72184, W00465, W38433, W44822, AA016301, AA033738, AA033739

T08T90" T28860

161	HAWBA28	171	351550	T86822, R18174, R42341, R54362, R54363, R42341, R72609, R72678, H19127, H19126, N30662, W52710, W61287, W65383, AA027835, AA027878, AA029269, AA053333, AA058647, AA114991, AA148794, AA148525, AA156780, AA156849
162	HSAAS44	172	670221	T79117, H09893, H10480, H86006, N40237, AA017440, AA132358, AA187785
162	HSAAS44	301	351553	T78632, T79117, R40722, R73748, H09723, H09804, H09893, H10480, H11320, H85873, H86006, N40237, N50800, N55157, AA017440, AA017441, AA029770, AA029769, AA031922, AA031993, AA088220, AA132238, AA132358, AA169653, AA169833, AA171744, AA171956, AA187785
163	HAFAL73	173	660102	R73802, H24981, H44193, H44192, H51123, AA234319
163	HAFAL73	302	351554	R73802, H24981, H44193, H44192, H51123
164	HSARF26	174	626839	T92615, H65155
164	HSARF26	303	361212	H65155
165	HEAAL31	175	639007	AA151656, AA151652, AA151733, AA151736
165	HEAAL31	304	361221	T56046, T56062, T56080, T56096, T90475, T90482, T90571, T90577, T74331, T89037, R10054, R11653, R54570, H22902, H24285, H24287, H50611, H50610, H67882, H67927, N27199, N36263, N39942, N40544, N44078, N46591, N58937, N73790, N94307, N95519, N99415, W02128, W19438, W32393, W57988, W58072, W73132, W90660, N90316, AA009898, AA022614, AA022615, AA025590, AA081613, AA084503, AA085189
166	HFKFX55	176	361222	R50327, N53166, N80292, N92405, N93437, W24630, W69468, AA013428, AA016267, AA132452, AA132636
167	H2LAO11	177	361224	T77193, R24954, R34527, R35684, R42813, R45217, R49037, R45217, R69485, R70378, H10333, H10332, R83327, R83424, H52395, H52396, H86524, H86605, H90386, H90437, H99168, N27057, N34842, N43917, N55352, N62688, N63631, N68510, N69539, N77359, N79085, N80462, N80477, W00825, W01972, W19934, W21605, W94195, AA018111, AA018112, AA040014, AA058863, AA063281, AA063266, AA086194, AA112364, AA112365, AA128903, AA134835
169	HPTTU11	179	653251	T47595, T47596, T74295, T90111, T82935, R34627, H40736, N39397, W45441, W45652, W44582, W44631, W47414, W47609, AA034253, AA035455, AA037139, AA059320, AA063639, AA226954, AA459767
169	HPTTU11	306	361247	T47596, T74295, T90111, T82935, H40736, N39397, W45441, W45652, W44582, W44631, W47414, W47609, AA034253, AA035455, AA037139, AA059320, AA059373, AA063639
170	HCFAR79	180	361318	T46931, T50673, T39801, T40801, T53401, T53402, R21169, R22220, R22275, R22787,

				R24273, R24382, R24908, R32461, R33226, R33227, R55432, R72796, R76252, R76535, R79988, R80091, R81411, R81412, R82779, R82786, H00624, H00630, H12733, H12734, H12738, H12739, H15831, H16139, H17120, H25890, H25891, H26787, H27500, H27572, H27734, H29299, H58288, H69891, H69892, H83814, H83952, H95016, H69892, H97790, N20245, N26081, N29310, N32053, N32279, N36118, N36720, N38746, N44124, N45024, N75811, N78841, N80850, W07334, W07515, W17199, W24810, W24844, W45102, W45152, W46243, W57629, W58578, W60475, W69835, W69945, W79132, W94310, W95456, W95564, N89582, AA032187, AA033661, AA036714, AA039761, AA053224, AA055646, AA057354, AA062720, AA075568, AA075604, AA079402, AA081343, AA081342, AA084535, AA086350, AA086062, AA086063, AA088496, AA088680, AA102649, AA101660, AA101659, AA114157, AA127925, AA131829, AA131995, AA143316, AA143333, AA143365, AA149758, AA156474, AA157072, AA157160, AA157305, AA157842, AA161119, AA161300, AA171710, AA179721, AA180474, AA181468, AA181640, AA181881, AA186914
172	HODCW06	182	361346	T57053, R94753, N57860, N64100
173	HFTAR26	183	361363	T89342, R32023, R32076, H00913, H73617, N30331, N30338, N56674, N99888, AA007590, AA055091, AA121507, AA127732
174	H2MBF44	184	663171	T51838, T51884, T92411, T65619, T75302, T80328, T81433, T81434, T96979, T97091, R00998, R07112, R13827, R13922, R35406, R39034, R49203, R53033, R49203, R61594, H00151, H01276, H05079, H07041, H14693, H16360, H38255, N21123, N31138, W47578, W47599, W56427, W90765, W90698, AA001816, AA004544, AA021298, AA081577, AA102004, AA112823, AA113108, AA137149, AA150268, AA150370, AA165473, AA181003, AA187989, AA259097
174	H2MBF44	308	361365	T51838, T51884, T92411, T65552, T65619, T75302, T80328, T81433, T81434, T96979, T97091, R00998, R07112, R13827, R13922, R27577, R27676, R35406, R37686, R38011, R38672, R39034, R49203, R51542, R53033, R49203, R61540, R61594, H00151, H01276, H05079, H07042, H07041, H14588, H14693, H16360, H37896, H38255, H99405, N21123, N31138, N34542, N75155, N80252, W07378, W42462, W45201, W47578, W47599, W56427, W74265, W79879, W90765, W90698, AA001746, AA001816, AA004544, AA021240, AA021298, AA069253, AA069252, AA081577, AA084448, AA086275, AA099494, AA102004, AA112823, AA113108, AA128022, AA135400, AA137149, AA150268, AA150370, AA165393, AA165473,

				AA181003, AA181189, AA182857, AA187989, AA187990, AA188268
175	HE8BI92	185	662220	R14311, R24143, R62968, R63250, R64668, R72480, R73256, R80927, H26458, R93403, R93450, H57481, H58002, H58409, H99384, N24149, N40700, N54068, N92774, W19427, W52093, W52957, W59973, W60435, AA099083, AA131685, AA131990, AA136214, AA136404, AA173574, AA173533, AA190478, AA191014
175	HE8BI92	309	361376	R14311, R24143, R62968, R63250, R64668, R72480, R73256, R80927, H26458, R93403, R93450, H57481, H58002, H58409, H99384, N24149, N40700, N54068, N92774, W19427, W52093, W52957, W59973, W60435, AA099083, AA131685, AA131990, AA136214, AA136404, AA158610, AA173574, AA173533, AA190478, AA191014
176	HFTBR48	186	661994	R10708, H98169, H99191, N21971, N24474, N28580, N29775, N32622, N47841, N49576, N57194, N71175, W19349, AA027991, AA191432, AA224194, AA224296, AA261907, AA262544, AA463932, AA464068
176	HFTBR48	310	361380	R10708, H98169, H99191, N21971, N24474, N28580, N29775, N32622, N47841, N49576, N57194, N71175, W19349, AA027991, AA191432
177	HE9CM64	187	605123	T53630, T53631, T75521, T78612, T79093, T85687, R19218, R44482, R44482, H03595, H28113, H41562, H46641, N33914, N80225, N93335, W38515, AA039786, AA039785, AA047032, AA047250, AA075305, AA463938, AA464013, AA464074, AA464722
177	HE9CM64	311	361382	T53630, T53631, T75521, T78612, T79093, T85687, R19218, R44482, R44482, H03595, H28113, H41562, H46641, N33914, N80225, N93335, W38515, AA039786, AA039785, AA047032, AA047250, AA075305
178	HATAV51	188	668035	T60208, T65846, T66000, R07954, R19068, R20084, R27055, R27054, R27789, R27878, R44110, R44110, R70833, R82660, R82711, H00472, H00526, H02416, H04214, H09792, H09878, H12753, H12795, H27575, R94131, H81849, H81850, H95618, N33235, N34207, N36442, N41951, N66133, N79969, N93411, W21452, W37638, W37856, W38928, W48827, W49808, W56224, W58537, W58503, W94176, AA025349, AA025357, AA039951, AA040002, AA055533, AA063282, AA158356, AA158429, AA158430, AA164832, AA166790, AA193584, AA193628, AA234543, AA236154, AA236123, AA429773
178	HATAV51	312	361385	T60208, T65846, T66000, R07954, R20084, R27055, R27054, R27789, R27878, R44110, R44110, R70781, R70833, R82660, R82711, H00472, H00526, H02416, H04214, H09792, H12753, H12795, R94131, H81849, H81850, H95618, N33235, N34207, N36442, N41951,

				N66133, N79969, N93411, W37375, W37638, W49808, W58503, AA025349, AA039951, AA055533, AA063282, AA158430, AA166675, AA166790, AA193584, AA193628
179	HAQAF27	189	361386	H27958, H27959, W86977, AA040831, AA043447, AA043448
180	HCEEK08	190	622186	N23815, N28919, W79250, AA025462, AA034130, AA262825
180	HCEEK08	313	361389	R38720, N22616, N63156, N63214, N79453, W74316, W76541, AA009980, AA022500, AA025463, AA039676, AA056428, AA173931
181	HAFAU18	191	666320	H20101, H40615, H49128, H50364, H50404, H51392, H99578, N23395, N24224, N24475, N26729, N28029, N29128, N34617, N35179, W38665, W44731, W92818, W92895, AA236416, AA236417, AA459710
181	HBGBT42	314	637497	H20101, H40615, H49128, H50364, H50404, H51392, N23395, N24224, N26729, N28029, N29128, N34617, N35179, W38665, W44731, W92895, AA236416, AA236417, AA459710
181	HAFAU18	315	361390	H99192, H99578, N21284, N35179, W92818, W92895, AA121981, AA121980
182	HETBY74	192	361396	R23770, R26913, R32172, R32216, R38317, R39395, R63720, R63767, R70906, R70940, R70993, R77169, R82584, R82585, H01942, H04495, H21696, H21906, H97740, H99748, N62092, N95055, W38893, W93362, W93394, W93606, AA016203, AA016243, AA076610, AA079633, AA079807, AA086218, AA158761, AA159815, AA159816, AA159710, AA159711, AA159565, AA160381, AA186897, AA188520
183	HTOAF35	193	661824	T39317, T48625, T48626, T51203, T51306, T51521, T51668, T51998, T52109, T53727, T54321, T57602, T91590, T91631, T92608, T92874, R44474, R44474, R72793, R75886, R76062, H01272, H28132, H41188, R86158, H51133, H51210, H85775, H92916, H95863, H95864, H98904, N23098, N24931, N25285, N29927, N29934, N40471, N48509, N53513, N66520, N70329, N75555, N92546, W02222, W04277, W05583, W39032, W84492, AA019752, AA043261, AA074472, AA076072, AA076029, AA079109, AA079148, AA088186, AA088656, AA099308, AA099818, AA126104, AA130889, AA130784, AA149583, AA165424, AA165588, AA169434, AA173473, AA173474, AA187661, AA186720
183	HTOAF35	316	361392	T39317, T48626, T51203, T51306, T51521, T51668, T51998, T52109, T53727, T54321, T57602, T91590, T91631, T92608, T92874, R44474, R44474, R72793, R75886, R76062, H01272, H28132, H41188, R86158, H51133, H51210, H85775, H92916, H95863, H95864, N23098, N24931, N25285, N29927, N29934, N40471, N48509, N53513, N66520, N70329, N75555, N92546, W02222, W04277, W05583,

				W39032, W84492, AA019752, AA043261, AA074472, AA076072, AA076029, AA079109, AA088186, AA088656, AA099308, AA099818, AA126104, AA130889, AA130784, AA149583, AA165424, AA165588, AA169434, AA173473, AA173474, AA187661, AA186720
184	HCRBX32	194	495055	T61851, T72629, T77182, T88844, R10094, R10194, T82253, T83530, R01229, R01343, R20727, R24715, R31457, R31458, R32713, R32714, R39272, R39439, R45449, R51552, R51553, R54400, R54499, R41197, R20727, R45449, R59399, R59459, H14539, H15683, H19939, H20326, H22915, H24093, H43536, R98988, R99766, H48909, H48910, H72736, H72735, N20635, N29007, N31914, N41658, N42699, N57556, N64823, N67014, N67536, N67783, N75675, N95768, W20500, W19698, W24881, W78750, W80488, W86081, N89954, AA015900, AA015899, AA046100, AA045995, AA099206, AA253083, AA253253, AA256913, AA255489
184	HCRBX32	317	361397	T61851, T72629, T72699, T77182, T88844, R10094, R10194, T82253, T83530, R01229, R01343, R20727, R24715, R31457, R31458, R32713, R32714, R39272, R39439, R41197, R45449, R51552, R51553, R54400, R54499, R41197, R20727, R45449, R59399, R59459, H14539, H15683, H19939, H20326, H22915, H24093, H43536, R98988, R99766, H48909, H48910, H72736, H72735, N20635, N29007, N31914, N41658, N42699, N57556, N64823, N67014, N67536, N67783, N75675, N95768, W20500, W19698, W24881, W78750, W80488, W86081, N89954, AA015900, AA015899, AA046100, AA045995, AA099206
185	HEBGB80	195	636011	R53950, W68693, W68786
185	HEBGB80	318	361398	R53950, W68693, W68786
186	HFAMH74	196	638302	R68577, R77269, H22571, H45827, H48519, H48687, N73815, N78323, W69528, AA122266, AA126601
186	HFAMH74	319	361402	R68372, R68577, R77269, R77319, H12718, H12719, H22571, H44679, H45826, H45827, H45875, H45877, R87058, H48519, H48687, H51661, H65843, H65930, H66425, H66424, H69570, H70028, N39190, N47250, N62091, N73815, N78323, W69445, W69528, AA122266, AA126601, AA131324, AA131790, AA131840, AA136024

"051301" T28860

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited</u>
	<u>Plasmid</u>	
20	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
	Zap Express	pBK
	lasmid BA	plasmid BA
	pSport1	pSport1
25	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
	pCR [®] 2.1	pCR [®] 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res.

17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR[®]2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample

09332171.061801
108T90.1228860

may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P - γ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl_2 , 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR

product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X.,
5 according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by,
10 among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P³² using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The
15 purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to
20 manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

Example 4: Chromosomal Mapping of the Polynucleotides

25 An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C.
30 Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose

gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

5 A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product
10 into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning
15 sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which
20 expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid
25 culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to
30 increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic

agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA

insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

- 5 The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

- 10 The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

- 15 Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

- 20 The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

- 25 The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

- 30 Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μ m membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 μ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus

Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40")

is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation

mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

5 Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a
10 microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is
15 then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life
20 Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a
25 micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

30 To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of

09682171.051801

infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of ^{35}S -methionine and 5 uCi ^{35}S -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a

selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide.

Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

5 The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

10 Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are
15 seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50
20 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed,
25 for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For
30 example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to

IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

```
GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCCACC
GTGCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTACACATGCG
TGGTGGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
```


GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT
 CCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
 GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAA
 CCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCG
 5 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC
 GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCAC
 CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGA
 TGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
 CCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

10

Example 10: Production of an Antibody from a Polypeptide

The antibodies of the present invention can be prepared by a variety of
 methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells
 expressing a polypeptide of the present invention is administered to an animal to
 15 induce the production of sera containing polyclonal antibodies. In a preferred
 method, a preparation of the secreted protein is prepared and purified to render it
 substantially free of natural contaminants. Such a preparation is then introduced into
 an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are
 20 monoclonal antibodies (or protein binding fragments thereof). Such monoclonal
 antibodies can be prepared using hybridoma technology. (Köhler et al.,
 Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al.,
 Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-
 Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures
 25 involve immunizing an animal (preferably a mouse) with polypeptide or, more
 preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in
 any suitable tissue culture medium; however, it is preferable to culture cells in Earle's
 modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at
 about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids,
 30 about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma
 cell line. Any suitable myeloma cell line may be employed in accordance with the

09332171.061801

present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma
5 cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is
10 possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the
15 polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies of the present invention may be used according to the methods disclosed
20 herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use
25 "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al.,
30 BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson

et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Example 11: Production Of Secreted Protein For High-Throughput

5 Screening Assays

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for
10 a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and
15 plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2×10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

20 The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom
25 plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

30 Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates

of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl₂ (anhyd); 0.00130 mg/L CuSO₄·5H₂O; 0.050 mg/L of Fe(NO₃)₃·9H₂O; 0.417 mg/L of FeSO₄·7H₂O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl₂; 48.84 mg/L of MgSO₄; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO₃; 62.50 mg/L of NaH₂PO₄·H₂O; 71.02 mg/L of Na₂HPO₄; .4320 mg/L of ZnSO₄·7H₂O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H₂O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H₂O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H₂O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H₂O; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B₁₂; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed

with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

5 The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

10 On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

15 It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 12: Construction of GAS Reporter Construct

20 One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

25 GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with
30 IL-12. Stat5 was originally called mammary growth factor, but has been found at

higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- α , IFN- γ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

	<u>JAKs</u>					<u>STATS</u>	<u>GAS(elements) or ISRE</u>
	<u>Ligand</u>		<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	
	<u>IFN family</u>						
5	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g			+	+	-	1
	IL-10		+	?	?	-	1,3
	<u>gp130 family</u>						
10	IL-6 (Pleiotrophic)		+	+	+	?	1,3
	IL-11(Pleiotrophic)		?	+	?	?	1,3
	OnM(Pleiotrophic)		?	+	+	?	1,3
	LIF(Pleiotrophic)?		+	+	?	1,3	
	CNTF(Pleiotrophic)		-/+	+	+	?	1,3
15	G-CSF(Pleiotrophic)		?	+	?	?	1,3
	IL-12(Pleiotrophic)		+	-	+	+	1,3
	<u>g-C family</u>						
20	IL-2 (lymphocytes)		-	+	-	+	1,3,5
	IL-4 (lymph/myeloid)		-	+	-	+	6
	>>Ly6)(IgH)						
	IL-7 (lymphocytes)		-	+	-	+	5
	IL-9 (lymphocytes)		-	+	-	+	5
	IL-13 (lymphocyte)		-	+	?	?	6
25	IL-15		?	+	?	+	5
	<u>gp140 family</u>						
	IL-3 (myeloid)		-	-	+	-	5
	(IRF1>IFP>>Ly6)						
30	IL-5 (myeloid)		-	-	+	-	5
	GM-CSF (myeloid)		-	-	+	-	5
	<u>Growth hormone family</u>						
35	GH		?	-	+	-	5
	PRL		?	+/-	+	-	1,3,5
	EPO		?	-	+	-	5
	CAS>IRF1=IFP>>Ly6)						GAS(B-
	<u>Receptor Tyrosine Kinases</u>						
40	EGF		?	+	+	-	1,3
	PDGF		?	+	+	-	1,3
	CSF-1		?	+	+	-	1,3

GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCCATTTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152),

although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4⁺ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 14: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2×10^7 U937 cells and

wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na₂HPO₄·7H₂O, 1 mM MgCl₂, and 675 uM CaCl₂. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting 1×10^8 cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of 5×10^5 cells/ml. Plate 200 ul cells per well in the 96-well plate (or 1×10^5 cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor).

The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

5 The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

10 5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

15 To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

20 PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

25 Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

30 To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS

(Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5×10^5 cells/ml.

Add 200 μ l of the cell suspension to each well of 96-well plate (equivalent to 1×10^5 cells/well). Add 50 μ l supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ μ l of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

Example 16: High-Throughput Screening Assay for T-cell Activity

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTC
CGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATG
GCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGC
AAAAAGCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

Example 17: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	(ml)	Rxn buffer diluent (ml)	CSPD
10	60		3
11	65		3.25
12	70		3.5
13	75		3.75
14	80		4
15	85		4.25
16	90		4.5

17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

5 Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes

in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to $2-5 \times 10^6$ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1×10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca⁺⁺ concentration.

Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar

Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

5 To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 μ l of the supernatant produced in Example 11, the medium was removed and 100 μ l of extraction buffer ((20 mM
10 HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na₃VO₄, 2 mM Na₄P₂O₇ and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 μ m membrane
15 bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

20 Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a
25 biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in
30 order. First, add 10 μ l of 5 μ M Biotinylated Peptide, then 10 μ l ATP/Mg₂⁺ (5 mM ATP/50 mM MgCl₂), then 10 μ l of 5x Assay Buffer (40 mM imidazole hydrochloride,

pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl₂, 5 mM MnCl₂, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

5 The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the
10 biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and
15 incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

20 **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be
25 used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by
30 substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are

then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre

Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

5 PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

10 Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the
15 corresponding genomic locus.

20 Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and
25 translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

30 A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus,

it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 23: Formulation

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray.

"Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to

09982171.061803
T08190" T 2728866

modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable
 5 polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP
 10 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

15 Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE
 20 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than
 25 about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J.*
 30 *Med.* 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

09682171-061801

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes

(e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis.

Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered

5 separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be

10 administered in combination with the Therapeutics of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either

15 concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through

20 separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the

25 Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™

30 (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the

invention, include, but are not limited to, CRXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott); COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β -L-FD4C and β -L-FddC (WO 98/17281).

Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

09882171-061801

Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 α , MIP-1 β , etc., may also inhibit fusion.

Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide

reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 α , MIP-1 β , SDF-1 α , IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN- α 2a; antagonists of TNFs, NF κ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*, *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α -naphthoflavone (WO 98/30213); and antioxidants such as γ -L-glutamyl-L-cysteine ethyl ester (γ -GCE; WO 99/56764).

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

5 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™,

DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™,
10 RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™,
CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™,
FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™,
KETOCONAZOLE™, ACYCLOVIR™, FAMCICLOVIR™, PYRIMETHAMINE™,
LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™

15 (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any

20 combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment,

Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or

25 prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific

30 embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another

0988271.061801
"061801" 228860

specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with

5 PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

10 In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin,
15 fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

In other embodiments, Therapeutics of the invention are administered in combination with immunosuppressive agents. Immunosuppressive agents that may
20 be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the
25 Therapeutics of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT®
30 (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as

DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrexate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

5 In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™
10 (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In certain embodiments, the Therapeutics of the invention are administered
15 alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal,
20 etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid
25 derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

30 In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but

are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, (1991)); Sulphated

Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; 5 Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, (1990)); Gold Sodium 10 Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, (1992)); and metalloproteinase 15 inhibitors such as BB94.

Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an 20 integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminimidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; 25 Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

30 Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the

administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the

that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel,

but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is

contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example, Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouracil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example,

Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further

embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

5 In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more
10 radisotopes. Particularly preferred isotopes are ⁹⁰Y and ¹¹¹In.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-
15 alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In one embodiment, the Therapeutics of the invention are administered in
20 combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma
25 (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No.
30 WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202),

312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor

(GM-CSF) (sargramostim, LEUKINE™, PROKINET™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCRIT™), stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

In certain embodiments, Therapeutics of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

In another embodiment, the Therapeutics of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amiodarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

In another embodiment, the Therapeutics of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorophenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport (e.g., furosemide, bumetanide, azosemide, piretanide, tripamide, ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).

In one embodiment, the Therapeutics of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, ^{127}I , radioactive isotopes of iodine such as ^{131}I and ^{123}I ; recombinant growth hormone, such as HUMATROPE™ (recombinant somatropin); growth

- hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and
- 5 METRODIN™ (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing
- 10 hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T₄™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T₃™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-
- 15 *n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca²⁺ channel blockers; dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).
- 20 Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™
- 25 (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™
- 30 (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and

09882171 061801

AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™ (mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus mestranol), PROGESTASERT™ (intrauterine device that releases progesterone),

5 LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone), LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-

10 NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

Additional treatments for endocrine and/or hormone imbalance disorders

15 include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™ (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™

20 and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids

25 and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™ (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE

30 SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and

VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate),

5 HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON

10 LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and

15 FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate),

20 DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and

25 ARISTOSPAN™ (triamcinolone hexacetonide); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to bovine, porcine or human insulin or mixtures thereof;

30 insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™;

oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™ (acetohexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™ (glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™

5 (gliclazide), GLUCOPHAGE™ (metformin), PRECOSE™ (acarbose), AMARYL™ (glimepiride), and ciglitazone; thiazolidinediones (TZDs) such as rosiglitazone, AVANDIA™ (rosiglitazone maleate) ACTOS™ (pioglitazone), and troglitazone; alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™
10 (diazoxide). In still other embodiments, Therapeutics of the invention are administered in combination with one or more of the following: a biguanide antidiabetic agent, a glitazone antidiabetic agent, and a sulfonylurea antidiabetic agent.

In one embodiment, the Therapeutics of the invention are administered in
15 combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN® (medroxyprogesterone), MICRONOR® (norethidrone acetate), PROMETRIUM®
20 progesterone, and megestrol acetate); and estrogen/progesterone combination therapies such as, for example, conjugated estrogens/medroxyprogesterone (e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estradiol (e.g., FEMHRT™).

In an additional embodiment, the Therapeutics of the invention are
25 administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B₁₂,
30 cyancobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin,

folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

In certain embodiments, the Therapeutics of the invention are administered in combination with agents used to treat psychiatric disorders. Psychiatric drugs that

may be administered with the Therapeutics of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine, divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g., amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranylcypromine, trazodone, trimipramine, and venlafaxine), antianxiety agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

In other embodiments, the Therapeutics of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents

that may be administered with the Therapeutics of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g., levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, ropinirole, pramipexole, benztropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

In another embodiment, Therapeutics of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents.

Vasodilating agents that may be administered with the Therapeutics of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, cilazapril, enalapril, enalaprilat,

fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 24: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

Example 25: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically

effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

Example 26: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge.

The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and

initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a

promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide

sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂ HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10⁶ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5X10⁶ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but

the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 28: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide.

The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., *Pharmacol. Res.* 35(6):517-522 (1997); Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express

polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA

in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

Example 29: Transgenic Animals.

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into

enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also

be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 30: Knock-Out Animals.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene

of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & 5 Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered 10 to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, 15 muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, *e.g.*, by transduction (using viral vectors, and preferably vectors that integrate the 20 transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The 25 engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, *e.g.*, in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered fibroblasts can be implanted as part of a skin graft; 30 genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and

09882171.061801

Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 31: Production of an Antibody

Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10

g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

*Isolation Of Antibody Fragments Directed polypeptide(s) of the invention
From A Library Of scFvs*

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

*Isolation Of Antibody Fragments Directed polypeptide(s) of the invention
From A Library Of scFvs*

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 µg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may

impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of

SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 33: T Cell Proliferation Assay

Proliferation assay for Resting PBLs.

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ³H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control

mAb (B33.1) overnight at 4 °C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4 /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 °C, plates are spun for 2 min. at 1000 rpm and 100 microliters of supernatant is removed and stored -20 °C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0.5 microcuries of ^3H -thymidine and cultured at 37 °C for 18-24 hr. Wells are harvested and incorporation of ^3H -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood lymphocytes) is measured by the up-take of ^3H -thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (*), IFN γ , TNF α , IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^3H -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of

proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

(*) The amount of the control cytokines IL-2, IFN γ , TNF α and IL-10 produced in each transfection varies between 300pg to 5ng/ml.

5

Costimulation assay.

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution,

therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFN γ , TNF α , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^3H -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

Costimulation assay: IFN γ and IL-2 ELISA

The assay is performed as follows. Twenty-four well plates are coated with either 300ng/ml or 600ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 500ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the costimulation assay. The assay is performed in the pre-coated twenty-four well plate using 1×10^5 cells/well in a final volume of 900ul. The supernatants (293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 300ul are added to 600ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only(negative control), IL-2, IFN γ , IL-12 and IL-18. In addition to the control supernatants recombinant human IL-2 (all cytokines were purchased from R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml, IL-12 at a final concentration of 1ng/ml and IL-18 at a final concentration of 50ng/ml are also used. Controls and unknown samples are tested in duplicate. Supernatant samples (250ul) are collected 2 days and 5 days after the beginning of the

assay. ELISAs to test for IFN and IL-2 secretion are performed using kits purchased from R & D Systems, (Minneapolis, MN). Results are expressed as an average of duplicate samples plus or minus standard error.

5 Proliferation assay for preactivated-resting T cells.

A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then
 10 cultured in the presence of low doses of IL-2 resemble effector T cells. These cells are generally more sensitive to further activation induced by growth factors such as IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would have an effect on a naïve T cell. Therefore the use of this type of cells might enable
 15 to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve) T cells.

The assay is performed as follows. PBMC are isolated by F/H gradient centrifugation from human peripheral blood, and are cultured in 10% FCS (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the
 20 presence of 2ug/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in 10% FCS/RPMI in the presence of 5ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are washed and rested in starvation medium (1%FCS/RPMI) for 16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to
 25 determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of in 10% FCS/RPMI containing the cells. Control
 30 supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFN , TNF , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 at a final concentration of 10ng/ml is

also used. After 24 hours of culture, each well is pulsed with 1uCi of ^3H -thymidine(Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- α , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC γ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune

response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ($10^6/\text{ml}$) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell

5 cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion

10 molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation.

15 Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium

20 azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that

25 activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral

30 blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque

05882171.061801

gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively

5 lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free
10 medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5 µg/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA
15 fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages

is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is
20 performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h
25 and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2×10^5

30 cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is

removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H₂O₂ produced by the macrophages, a standard curve of a H₂O₂ solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 35: Biological Effects of Polypeptides of the Invention

Astrocyte and Neuronal Assays

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to

induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE₂ assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 α for 24 hours. The supernatants are collected and assayed for PGE₂ by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic

projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP⁺) and released.

- 5 Subsequently, MPP⁺ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP⁺ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

10 It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J.

- 15 Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential
20 effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm² on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium
25 containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

- 30 Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons

would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2.5×10^4 cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was

performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF₁₆₅ or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida *et al.*, J. Biol. Chem. 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

Example 39: Stimulation of Endothelial Migration

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

10 Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate
15 concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5×10^5 cells suspended in 50 ul M199 containing 1%
20 FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO₂ to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by
25 counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

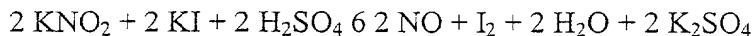
 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the
30 invention.

Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of KNO_2 (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H_2SO_4 . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions.

S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per 1×10^6 endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The

ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese qual (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old qual embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse

In vivo angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the

mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 44: Rescue of Ischemia in Rabbit Lower Limb Model

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.*, *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.*, *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1

min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

Example 45: Effect of Polypeptides of the Invention on Vasodilation

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as $p < 0.05$ vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 46: Rat Ischemic Skin Flap Model

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of

polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

Ischemic skin

5 Ischemic skin wounds

Normal wounds

The experimental protocol includes:

Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

10 An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

15 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

20 **Example 47: Peripheral Arterial Disease Model**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

25 One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

30 The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

T0829071.061801

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

Example 48: Ischemic Myocardial Disease Model

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

10

The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

15

a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

20

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 49: Rat Corneal Wound Healing Model

25

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

Inserting a spatula below the lip of the incision facing the outer corner of the eye.

Making a pocket (its base is 1-1.5 mm from the edge of the eye).

30

Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

09882171.061801

Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

Diabetic db+/db+ Mouse Model.

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These

homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

09992171.061801

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm², the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary

antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad*

libitum. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

5 The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue
10 punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

 Wounds are visually examined and photographed at a fixed distance at the day of
15 wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

 The polypeptide of the invention is administered using at a range different doses,
20 from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

 Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue
25 cassettes between biopsy sponges for further processing.

 Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

 Wound closure is analyzed by measuring the area in the vertical and horizontal
30 axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8).

The wound area on day 1 is 64mm², the corresponding size of the dermal punch.

Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 51: Lymphadema Animal Model

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing.

Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then
 5 dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca²⁺ comparison.

10 Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

15 Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to
 20 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention

25 The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and
 30 endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and

extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- α), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- α induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- α treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO₂.

HUVECs are seeded in 96-well plates at concentrations of 1×10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 μ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 μ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution

of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with

5 PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is
10 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [
15 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the
20 invention.

Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell

Proliferation

This assay is based on the ability of human CD34+ to proliferate in the
25 presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range
30 of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone

has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL-3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5×10^5 cells/ml. During this time, 100 μ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μ l of prepared cytokines, 50 μ l SID (supernatants at 1:2 dilution = 50 μ l) and 20 μ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μ l. The plates are then placed in a 37°C/5% CO₂ incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μ Ci/well of [3H] Thymidine is added in a 10 μ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined

via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the “Immune Activity” and “Infectious Disease” sections above, and elsewhere herein.

Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The

factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with
 5 fn fragment at a coating concentration of $0.2 \mu\text{g}/\text{cm}^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in
 10 the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2 , 7% O_2 , and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA.

15 Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or
 20 antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and
 25 "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the
 30 proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses

of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and stenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without TNF α stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) 2000 cells/well (AoSMC) in 100 μ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 5% FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μ g/ml hFGF, 5mg/ml insulin, 1 μ g/ml hFGF, 50mg/ml gentamycin, 50 μ g/ml Amphotericin B, 5% FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains Clonetics FB basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for

AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls.

5. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO₂ until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

- 15 On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 µl/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

- 25 Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100 µl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or

smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vascularogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of

the ExtrAvidin-Alkaline Phosphatase in glycine buffer: $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$. 5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNPN reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 57: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The

plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM[®], density 1.0770 g/ml,

Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 μ C of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

Table 3

	Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Met 1	A		.					1.30	-0.61				0.95	2.10
	Arg 2	.		B	.				0.88	-1.04	*			0.95	3.21
	Lys 3			B	B				0.98	-0.79	*			0.75	2.07
	Thr 4	A	.	.	B				1.02	-0.30	.			0.45	2.20
	Arg 5	.		B	B				0.60	-0.49	.		F	0.60	1.11
10	Leu 6	A	.	.	B				0.39	0.20	*			-0.30	0.46
	Trp 7			B	B				-0.01	0.89	*			-0.60	0.26
	Gly 8	A	.	.	B				-0.66	1.31	.	*		-0.60	0.14
	Leu 9	A	.	.	B				-1.16	1.93	*			-0.60	0.17
	Leu 10		.	B	B				-1.97	1.93	*	*		-0.60	0.13
15	Trp 11	.		B	B				-2.01	1.80	.			-0.60	0.12
	Met 12		.	B	B				-2.02	2.01	.			-0.60	0.10
	Leu 13	A	.	.	B				-1.68	1.71	.			-0.60	0.17
	Phe 14	A	.	.	B				-1.68	1.03	*	*		-0.60	0.28
	Val 15	A	.	.	B				-0.76	0.80	*	*		-0.60	0.23
20	Ser 16	A	A	.					-1.06	0.19	*	*		-0.30	0.55
	Glu 17	A	A	.					-1.04	0.00	.	*		-0.30	0.64
	Leu 18	A	A	.					-0.54	-0.29	*	*		0.30	0.88
	Arg 19	A	A	.					0.20	-0.44	*	*		0.30	0.94
	Ala 20	A	A	.					0.24	-0.83	*	*		0.75	1.09
25	Ala 21	A	A	.					0.23	-0.14	*	*		0.45	1.09
	Thr 22	A	A	.					0.23	-0.34	*	*	F	0.45	0.80
	Lys 23	A	A	.					1.04	-0.34	*	*	F	0.60	1.38
	Leu 24	A	A	.					0.98	-0.84	*	*	F	0.90	2.36
	Thr 25	A	A	.					1.32	-1.34	.		F	0.90	3.27
30	Glu 26	A	A	.					1.91	-1.07	.		F	0.90	2.56
	Glu 27	A	A	.					1.41	-1.07	.	*	F	0.90	5.38
	Lys 28	A	A	.					1.41	-1.07	.	*	F	0.90	3.08
	Tyr 29	A	A	.					2.22	-1.56	.	*	F	0.90	3.55
	Glu 30	A	A	.					2.19	-1.56	.	*	F	0.90	3.55
35	Leu 31	A	A	.					2.19	-1.13	.	*	F	0.90	1.76
	Lys 32	A	A	.					1.88	-0.73	*	*	F	0.90	1.94
	Glu 33	A	A	.					1.02	-1.00	*	*	F	0.90	1.62
	Gly 34	A	A	.					1.27	-0.31	.	*	F	0.60	1.62
	Gln 35	A	A	.					0.41	-1.00	.	*	F	0.90	1.35
40	Thr 36	A	A	.					1.27	-0.36	.	*	F	0.45	0.58
	Leu 37	A	A	.					0.56	-0.36	.	*	F	0.60	1.17
	Asp 38	.	A	B	.				0.56	-0.21	.	*	.	0.30	0.36
	Val 39	.	A	B	.				0.66	-0.61	.	*	.	0.60	0.42
	Lys 40	.	A	B	.				0.34	-0.34	.	*	.	0.30	0.80
45	Cys 41	.	.	B	.		T		-0.16	-0.54	.	*	.	1.00	0.69
	Asp 42	A	.	.	.		T		0.66	0.14	.	*	.	0.10	0.77
	Tyr 43	A	.	.	.		T		0.70	-0.50	.	*	.	0.70	0.66
	Thr 44	A	.	.	.		T		0.86	-0.50	*	*	.	0.85	2.47
	Leu 45	A	A	.					0.22	-0.29	*	*	.	0.45	1.28
50	Glu 46	A	A	.					0.59	0.21	*	.		-0.30	0.83
	Lys 47	A	A	.					0.29	-0.16	.	.		0.30	0.77
	Phe 48	A	A	.					0.53	-0.26	*	.	F	0.60	1.25
	Ala 49	A	.	.			T		0.89	-0.54	.	.	F	1.30	1.25
	Ser 50	A	.	.			T		1.11	-0.54	*	.	F	1.30	1.25
55	Ser 51	A	.	.			T		0.82	-0.04	.	.	F	1.00	1.46
	Gln 52	A	.	.			T		0.78	0.09	.	*	F	0.40	1.51
	Lys 53	A	.	.					0.59	-0.01	*	.	F	0.80	1.96
	Ala 54	A	.	.	B				0.29	0.29	*	.	.	-0.15	1.02
	Trp 55	A	.	.	B				0.70	0.59	*	.	.	-0.60	0.41
60	Gln 56	.	.	B	B				1.00	0.19	*	.	.	-0.30	0.41
	Ile 57	.	.	B	B				0.66	0.19	*	.	.	0.04	0.67
	Ile 58	.	.	B	.		T		0.61	0.11	*	.	.	0.78	0.63
	Arg 59	.	.	B	.		T		0.60	-0.80	*	.	.	2.02	0.63

051801 1228860

5	Asp	60	T	T	.	0.68	-0.59	*	.	F	2.91	0.89
	Gly	61	T	T	.	0.72	-0.84	*	.	F	3.40	1.97
	Glu	62	C	1.30	-1.53	*	.	F	2.66	2.01
	Met	63	T	C	1.38	-1.04	.	.	F	2.52	1.74
	Pro	64	T	T	.	0.68	-0.36	*	.	F	2.08	1.45
10	Lys	65	T	T	.	0.01	-0.29	.	.	F	1.59	0.84
	Thr	66	A	T	.	0.04	0.29	.	.	F	0.25	0.46
	Leu	67	A	A	0.04	0.16	*	.	.	-0.30	0.43
	Ala	68	A	A	0.76	-0.27	*	.	.	0.30	0.37
	Cys	69	A	A	0.76	-0.27	*	.	.	0.30	0.50
15	Thr	70	A	A	0.41	-0.33	*	.	.	0.64	0.94
	Glu	71	A	0.77	-0.63	*	.	F	1.78	1.25
	Arg	72	A	1.58	-1.13	*	.	F	2.12	4.65
	Pro	73	T	.	.	1.87	-1.30	.	.	F	2.86	5.18
	Ser	74	T	T	.	2.50	-1.40	*	.	F	3.40	4.01
20	Lys	75	T	T	.	2.60	-0.90	*	.	F	3.06	2.79
	Asn	76	T	T	.	1.74	-0.47	*	.	F	2.42	2.79
	Ser	77	T	C	1.63	-0.26	.	*	F	1.88	1.54
	His	78	.	.	B	B	.	.	.	0.99	-0.24	.	.	F	0.94	1.34
	Pro	79	.	.	B	B	.	.	.	0.94	0.40	.	.	.	-0.60	0.62
25	Val	80	.	.	B	B	.	.	.	1.01	0.43	.	*	.	-0.60	0.46
	Gln	81	.	.	B	B	.	.	.	0.12	0.04	*	.	.	-0.30	0.66
	Val	82	.	.	B	B	.	.	.	-0.47	0.23	*	.	.	-0.30	0.30
	Gly	83	.	.	B	B	.	.	.	-1.24	0.49	*	*	.	-0.60	0.28
	Arg	84	.	.	B	B	.	.	.	-1.03	0.53	*	.	.	-0.60	0.13
30	Ile	85	.	.	B	B	.	.	.	-0.18	0.13	*	.	.	-0.30	0.31
	Ile	86	.	.	B	B	.	.	.	-0.42	-0.51	*	.	.	0.60	0.53
	Leu	87	.	.	B	B	.	.	.	0.40	-0.19	*	*	.	0.30	0.42
	Glu	88	.	.	B	B	.	.	.	0.74	0.31	*	*	.	-0.30	0.82
	Asp	89	A	0.60	-0.37	*	*	.	0.65	1.95
35	Tyr	90	A	1.14	-0.56	.	.	.	0.95	3.21
	His	91	A	T	.	1.22	-0.81	.	.	.	1.15	1.84
	Asp	92	A	T	.	1.22	-0.13	.	*	.	0.70	0.91
	His	93	A	T	.	1.33	0.56	*	*	.	-0.20	0.48
	Gly	94	A	T	.	0.48	-0.20	.	*	.	0.70	0.69
40	Leu	95	A	.	.	B	.	.	.	0.83	-0.06	.	*	.	0.30	0.31
	Leu	96	A	.	.	B	.	.	.	0.27	-0.06	.	*	.	0.30	0.44
	Arg	97	.	.	B	B	.	.	.	-0.59	0.06	.	*	.	-0.30	0.44
	Val	98	.	.	B	B	.	.	.	-0.56	0.27	.	*	.	-0.30	0.40
	Arg	99	.	.	B	B	.	.	.	-1.02	-0.01	.	*	.	0.30	0.77
45	Met	100	.	.	B	B	.	.	.	-0.21	-0.01	.	*	.	0.30	0.32
	Val	101	.	.	B	B	.	.	.	-0.26	0.39	.	*	.	-0.30	0.76
	Asn	102	.	.	B	B	.	.	.	-0.37	0.39	.	*	.	-0.30	0.29
	Leu	103	.	.	B	B	.	.	.	0.49	0.39	*	*	.	-0.30	0.50
	Gln	104	.	.	B	B	.	.	.	0.08	-0.23	*	*	.	0.73	1.13
50	Val	105	.	.	B	B	.	.	.	0.33	-0.49	.	*	.	0.86	0.94
	Glu	106	.	.	B	B	.	.	.	0.38	-0.46	.	*	F	1.44	1.13
	Asp	107	T	T	.	0.13	-0.46	.	*	F	2.37	0.54
	Ser	108	T	T	.	0.94	-0.10	.	*	F	2.80	1.14
	Gly	109	T	T	.	0.28	-0.34	.	.	F	2.52	1.14
55	Leu	110	T	T	.	0.28	0.23	.	.	.	1.34	0.36
	Tyr	111	.	.	B	B	.	.	.	-0.61	0.87	.	.	.	-0.04	0.20
	Gln	112	.	.	B	B	.	.	.	-0.86	1.17	.	.	.	-0.32	0.14
	Cys	113	.	.	B	B	.	.	.	-0.56	1.50	.	.	.	-0.60	0.27
	Val	114	.	.	B	B	.	.	.	-0.42	1.21	.	.	.	-0.60	0.30
60	Ile	115	.	.	B	B	.	.	.	0.18	0.89	*	.	.	-0.26	0.27
	Tyr	116	.	.	B	0.47	0.91	*	.	.	0.28	0.77
	Gln	117	C	0.47	0.34	*	.	.	1.27	2.09
	Pro	118	T	C	0.92	-0.30	*	.	F	2.56	5.15
	Pro	119	T	T	.	1.74	-0.56	*	.	F	3.40	5.08
60	Lys	120	T	C	2.03	-0.81	*	.	F	2.86	3.99
	Glu	121	T	C	1.47	-0.60	*	.	F	2.52	2.56
	Pro	122	A	0.77	-0.34	*	.	F	1.48	1.36

5	His	123	A	.	.	B	.	.	0.98	0.01	*	.	0.04	0.59
	Met	124	A	.	.	B	.	.	1.30	0.01	*	.	-0.30	0.57
	Leu	125	A	.	.	B	.	.	0.37	0.01	*	*	-0.30	0.72
	Phe	126	A	.	.	B	.	.	0.48	0.27	*	*	-0.30	0.37
	Asp	127	A	.	.	B	.	.	-0.12	-0.23	*	*	0.30	0.74
10	Arg	128	A	.	.	B	.	.	-0.94	-0.16	*	*	0.30	0.74
	Ile	129	A	.	.	B	.	.	-1.20	-0.20	*	*	0.30	0.63
	Arg	130	.	.	B	B	.	.	-0.70	-0.34	*	*	0.30	0.28
	Leu	131	.	.	B	B	.	.	0.04	0.14	*	*	-0.30	0.21
	Val	132	.	.	B	B	.	.	-0.30	0.14	*	*	-0.30	0.59
15	Vai	133	.	.	B	B	.	.	-1.11	-0.11	*	*	0.30	0.30
	Thr	134	.	.	B	B	.	.	-0.52	0.67	*	*	-0.45	0.31
	Lys	135	.	.	B	B	.	.	-0.98	0.37	.	*	-0.15	0.56
	Gly	136	.	.	B	.	T	.	-0.48	0.16	.	F	0.25	0.75
	Phe	137	.	.	B	.	T	.	0.17	0.00	.	F	0.25	0.75
20	Ser	138	.	.	B	.	T	.	0.68	-0.06	.	F	0.85	0.58
	Gly	139	T	C	0.69	0.37	.	F	0.45	0.58
	Thr	140	T	C	0.64	0.33	.	F	0.45	0.90
	Pro	141	T	C	0.99	-0.06	.	F	1.20	1.08
	Gly	142	T	C	1.69	-0.44	.	F	1.54	1.89
25	Ser	143	T	C	1.69	-0.47	.	F	1.88	2.11
	Asn	144	T	C	1.72	-0.57	.	F	2.52	1.82
	Glu	145	T	C	2.03	-0.51	.	F	2.86	2.66
	Asn	146	T	T	2.24	-0.54	.	F	3.40	3.44
	Ser	147	T	T	1.73	-0.53	.	F	3.06	3.44
30	Thr	148	.	.	.	B	T	.	1.79	-0.29	*	.	2.02	1.47
	Gln	149	.	.	B	B	.	.	1.83	0.47	*	.	0.38	1.44
	Asn	150	.	.	B	B	.	.	0.94	0.07	*	.	0.34	2.14
	Val	151	.	.	B	B	.	.	0.73	0.37	*	.	0.00	1.04
	Tyr	152	.	.	B	B	.	.	0.82	0.31	*	.	-0.30	0.93
35	Lys	153	.	.	B	B	.	.	0.82	0.34	*	.	-0.15	0.89
	Ile	154	.	.	B	B	.	.	0.51	0.43	*	.	-0.30	1.74
	Pro	155	.	.	B	.	.	T	0.20	0.27	*	.	0.40	1.60
	Pro	156	T	T	1.10	0.00	*	.	0.80	1.15
	Thr	157	T	T	0.76	0.00	*	.	0.80	3.29
40	Thr	158	.	.	B	.	.	T	-0.10	-0.19	*	.	1.00	2.15
	Thr	159	.	A	B	.	.	.	0.12	0.07	*	.	0.00	1.15
	Lys	160	.	A	B	.	.	.	0.12	0.21	*	.	-0.15	0.43
	Ala	161	.	A	B	.	.	.	-0.48	0.16	*	.	-0.30	0.46
	Leu	162	.	A	B	.	.	.	-0.41	0.36	*	.	-0.30	0.26
45	Cys	163	.	.	B	.	.	T	-0.41	0.63	*	.	-0.20	0.20
	Pro	164	.	.	B	.	.	T	-0.40	1.11	*	.	-0.20	0.29
	Leu	165	.	.	B	.	.	T	-0.66	1.00	*	*	-0.20	0.47
	Tyr	166	.	.	B	.	.	T	0.04	0.74	*	*	-0.05	1.37
	Thr	167	.	.	B	.	.	.	0.54	0.17	*	.	0.20	1.74
50	Ser	168	T	0.36	0.23	*	.	0.60	3.04
	Pro	169	.	.	B	.	.	T	0.26	0.19	*	.	0.40	1.44
	Arg	170	.	.	B	.	.	T	1.07	-0.09	*	*	1.00	1.44
	Thr	171	.	.	B	.	.	T	0.72	-0.17	*	.	1.00	1.86
	Val	172	.	.	B	B	.	.	0.82	-0.06	*	.	0.60	1.21
55	Thr	173	.	.	B	B	.	.	0.91	-0.06	*	*	0.73	0.96
	Gln	174	.	.	B	B	.	.	1.17	0.37	*	*	0.56	1.03
	Ala	175	.	.	B	B	.	.	0.76	-0.11	*	*	1.44	2.77
	Pro	176	T	0.76	-0.37	.	.	2.32	2.57
	Pro	177	T	1.02	-0.37	.	*	2.80	2.14
60	Lys	178	T	1.33	-0.27	.	*	2.52	2.14
	Ser	179	.	.	B	.	.	T	0.48	-0.77	.	*	2.14	2.31
	Thr	180	.	.	B	B	.	.	0.77	-0.56	.	*	1.46	1.11
	Ala	181	.	.	B	B	.	.	0.67	-0.60	.	*	1.03	0.74
	Asp	182	.	.	B	B	.	.	0.67	-0.11	.	*	0.45	0.80
60	Val	183	.	.	B	B	.	.	0.62	-0.07	.	*	0.79	0.86
	Ser	184	.	.	B	.	.	.	0.62	-0.56	.	.	1.78	1.42
	Thr	185	.	.	B	.	.	T	0.93	-0.67	.	*	2.32	1.14

5	Pro	186	T	T	C	0.63	-0.67	.	*	F	2.86	2.66
	Asp	187	T	T	.	0.63	-0.63	.	*	F	3.40	1.39
	Ser	188	.	.	B	.	T	T	.	0.68	-0.61	.	*	F	2.66	1.55
	Glu	189	.	.	B	0.67	-0.41	.	*	F	1.67	0.83
	Ile	190	.	.	B	0.98	-0.36	.	*	F	1.33	0.71
10	Asn	191	.	.	B	0.33	0.04	.	*	.	0.24	0.86
	Leu	192	.	.	B	B	.	.	.	0.02	0.30	.	*	.	-0.30	0.37
	Thr	193	.	.	B	B	.	.	.	0.32	0.79	.	*	F	-0.60	0.76
	Asn	194	.	.	B	B	.	.	.	-0.57	0.10	*	*	F	-0.15	0.78
	Val	195	.	.	B	B	.	.	.	-0.57	0.39	*	*	F	-0.15	0.67
15	Thr	196	.	.	B	B	.	.	.	-0.46	0.39	*	*	F	-0.15	0.32
	Asp	197	.	.	B	B	.	.	.	-0.50	-0.10	*	*	.	0.30	0.39
	Ile	198	.	.	B	B	.	.	.	-0.40	0.14	*	*	.	-0.30	0.39
	Ile	199	.	.	B	B	.	.	.	-1.26	-0.07	*	*	.	0.30	0.42
	Arg	200	.	.	B	B	.	.	.	-1.10	0.09	*	*	.	-0.30	0.19
20	Val	201	.	.	B	B	.	.	.	-0.79	0.87	*	.	.	-0.60	0.23
	Pro	202	.	.	B	B	.	.	.	-1.68	0.59	*	.	.	-0.60	0.53
	Val	203	.	.	B	B	.	.	.	-1.64	0.59	.	*	.	-0.60	0.19
	Phe	204	.	.	B	B	.	.	.	-1.64	1.23	.	*	.	-0.60	0.19
	Asn	205	.	.	B	B	.	.	.	-2.57	1.27	.	*	.	-0.60	0.09
25	Ile	206	.	.	B	B	.	.	.	-2.52	1.53	.	.	.	-0.60	0.10
	Val	207	.	.	B	B	.	.	.	-2.90	1.57	.	.	.	-0.60	0.09
	Ile	208	.	.	B	B	.	.	.	-2.39	1.29	.	.	.	-0.60	0.06
	Leu	209	.	.	B	B	.	.	.	-2.03	1.31	.	*	.	-0.60	0.08
	Leu	210	.	.	B	.	.	T	.	-2.73	1.06	.	.	.	-0.20	0.11
30	Ala	211	.	.	B	.	.	T	.	-2.66	1.20	.	.	.	-0.20	0.13
	Gly	212	A	T	.	-2.10	1.20	.	.	.	-0.20	0.13
	Gly	213	A	T	.	-1.17	0.90	.	.	.	-0.20	0.22
	Phe	214	A	-0.66	0.21	.	.	.	-0.10	0.43
	Leu	215	A	-0.66	0.10	.	.	F	0.05	0.58
35	Ser	216	.	.	B	.	.	T	.	-0.92	0.36	.	.	F	0.25	0.48
	Lys	217	.	.	B	.	.	T	.	-1.28	0.57	.	.	F	-0.05	0.42
	Ser	218	.	.	B	.	.	T	.	-1.23	0.57	.	.	F	-0.05	0.44
	Leu	219	.	.	B	.	.	T	.	-1.39	0.27	.	.	.	0.10	0.44
	Val	220	.	.	B	B	.	.	.	-1.39	0.53	.	.	.	-0.60	0.16
40	Phe	221	.	.	B	B	.	.	.	-1.79	1.21	.	.	.	-0.60	0.10
	Ser	222	.	.	B	B	.	.	.	-2.42	1.61	.	.	.	-0.60	0.10
	Val	223	.	.	B	B	.	.	.	-2.98	1.43	.	.	.	-0.60	0.14
	Leu	224	.	.	B	B	.	.	.	-2.48	1.43	.	*	.	-0.60	0.12
	Phe	225	A	.	.	B	.	.	.	-2.43	1.13	*	*	.	-0.60	0.13
45	Ala	226	A	.	.	B	.	.	.	-1.62	1.43	*	*	.	-0.60	0.15
	Val	227	A	.	.	B	.	.	.	-1.62	0.79	*	*	.	-0.60	0.35
	Thr	228	.	.	B	B	.	.	.	-1.47	0.49	.	*	.	-0.60	0.54
	Leu	229	.	.	B	B	.	.	.	-1.51	0.49	*	*	.	-0.60	0.46
	Arg	230	.	.	B	B	.	.	.	-1.02	0.63	*	*	.	-0.60	0.46
50	Ser	231	.	.	B	B	.	.	.	-0.82	0.41	.	*	.	-0.60	0.49
	Phe	232	.	.	B	B	.	.	.	-0.36	0.36	.	*	.	-0.30	0.76
	Val	233	.	.	B	B	.	.	.	-0.43	0.10	*	*	.	-0.30	0.50
	Pro	234	.	.	B	B	.	.	.	-0.01	0.53	*	*	.	-0.60	0.48